# THE EFFECTS OF HEAVY METALS ON SEDIMENT MACROFAUNA

Pro gradu thesis

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Tiivistelmä – Referat – Abstract

Raskasmetallisaasteen aiheuttama ympäristön kuormittuminen voi uhata ekosysteemin toimintaa. Sedimentin makrofaunaa käytetään usein tutkittaessa ympäristön pitkän aikavälin kuormitusta, sillä siihen kuuluvat eliöt ovat suhteellisen hidasliikkeisiä tai paikallaanpysyviä ja heijastavat näin tutkittavalla alueella tavallisesti vallitsevia ympäristöoloja. Tämän tutkimuksen tavoitteena on selvittää, vaikuttavatko raskasmetallipäästöt läntisen Suomenlahden alueella sijaitsevan entisen Koverharin terästehtaan ympäristön makrofaunayhteisöön. Useita makrofaunayhteisöön ja lajirikkauteen perustuvia indeksejä käytetään ympäristön tilan kuvaamiseen. Tässä tutkimuksessa arvioidaan kolmen eri indeksin – Shannon-Wienerin indeksin (H'), Benthic Quality Indeksin (BQI) ja Brackish water Benthic Indeksin (BBI) – kykyä havainnoida raskasmetallien vaikutusta meriympäristöön. Tutkimus vertailee myös kahta makrofaunanäytteenottomenetelmää, GEMAX -putkinäytteenotinta ja van Veen -kauhanäytteenotinta selvittääkseen, onko niillä saatujen eliöyhteisönäytteiden välillä rakenteellisia eroja. Tutkimuksessa selvisi, että vaikka saastuneimmilla asemilla oli viitteitä ympäristökuormituksesta, kuten herkkien lajien puuttuminen ja stressiä kestävien lajen runsaat yksilömäärät, raskasmetallikuormitusta ei voitu kiistattomasti todistaa kuormituksen syyksi. H' ja BBI eivät havainneet asemien välillä raskasmetallikuormitukseen mahdollisesti liittyviä eroja, mutta BQI löysi osan makrofaunayhteisön analyysissä havaituista eroista. Näytteenottomenetelmien pyydystämien makrofaunayhteisöjen välillä ei havaittu merkittäviä eroja, mutta niistä laskettujen indeksien arvot poikkesivat toisistaan merkittävästi. Samalta näytteenottoasemalta otettujen GEMAX -näytteiden replikaattien välillä oli enemmän vaihtelua kuin verrattain yhdenmukaisissa van Veen -replikaatit.

Environmental stress caused by heavy metal contamination of the sediment can threaten ecosystem functioning. Sediment macrofauna are often used to study the effects of environmental stress factors over time, as they are relatively sedentary and thus reflect the ambient conditions in an area. This study investigates whether heavy metal pollution influences the macrofaunal community adjacent to a former steel works factory in Koverhar, in the western Gulf of Finland. Various indices based on macrofaunal community composition and diversity are used in the Baltic Sea to evaluate the environmental status. This thesis evaluates the performance of three of these indices, Shannon-Wiener's Index (H'), Benthic Quality Index (BQI) and Brackish water Benthic Index (BBI), in detecting the influence of heavy metal pollution on the marine environment. Two macrofaunal sampling methods, GEMAX corer and van Veen grab, are also compared to each other to investigate if there are differences in the structure of the macrofaunal communities that they capture. The study found that while there were indications of environmental stress, such as a lack of sensitive species and an abundance of tolerant species at the more heavily polluted stations, the heavy metal pollution could not be definitively proven to be the cause. H' and BBI failed to find the differences potentially associated with heavy metal pollution between the stations, while BQI detected some of the differences found by the macrofaunal community analysis. The two sampling methods were found to not be significantly different from each other in terms of macrofaunal communities, but yielded significantly different macrofaunal index values, with the GEMAX results displaying a larger variance between replicates while the van Veen results were more consistent.

Avainsanat – Nyckelord – Keywords

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Muita tietoja – Övriga uppgifter – Additional information

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# 1. Introduction

Heavy metals are generally defined as metals that are dense, or have high atomic weight, although their significance to biotic interactions is more related to their chemistry and interactions with other substances (Hawkes, 1997). Some metals classified as heavy by atomic weight (iron, selenium) are necessary for organisms in very small doses, but when talking of pollution, and within the context of this thesis, heavy metals refer to the more toxic variety like cadmium, mercury or lead (Bryan & Langston, 1992). Some heavy metals, like methylmercury, can bioaccumulate and reach dangerous concentrations in animals higher up in the trophic network (Bryan & Langston, 1992). Due to the danger the heavy metals pose to ecosystem functioning and possibly even humans, it is crucial to know if and to what extent a system is contaminated. Macrofauna can play an important role in examining this (Ryu et al., 2011).

The sediment macrofaunal community is comprised of relatively sedentary or slow-moving organisms that are for the most part heavily in contact and interacting with their substrate (Ryu et al., 2011). This makes them useful for studying the state of the marine environment, since they tend to reflect the ambient conditions in the sediment and respond to environmental stresses, like pollution, climate change and eutrophication (Ryu et al., 2011; Rousi et al., 2013). Previous studies have found that high heavy metal concentrations tend to cause a decline of species diversity, biomass and mean species size (Ryu et al., 2011). Typically a simultaneous increase is seen in the abundances of the species that can tolerate higher environmental stress, as they can now take advantage of the resources freed up by their less tolerant competitors (Ryu et al., 2011). Copper (Cu), nickel (Ni) and tin (Sn) may cause repressed growth and physical deformations, while cadmium (Cd), chrome (Cr), zinc (Zn), Cu and Sn may cause a decrease in fertility and / or survival of the young life stages (Bryan & Langston, 1992; Chandler et al., 2014). Cd has also been known to cause changes in metabolism (Bryan & Langston, 1992). Some heavy metals, such as arsenic (As) and tin (Sn), have been proven to bioaccumulate in the food web (Bryan & Langston, 1992). On the other hand, several species of bivalves and polychaeta have also shown the ability to develop higher tolerance to heavy metals if continuously exposed to them (Bryan & Langston, 1992).

Organic loading can influence the bioavailability of heavy metals and the distribution of macrofauna (Bryan & Langston, 1992). In the context of this study, organic loading refers to dead, nutrient-rich matter of organic origin, either natural or anthropogenic. While decomposing, it uses up dissolved oxygen (DO) from the water and sediment, releases organic carbon and nutrients into the system, and causes changes in the redox potential in water and sediments. These effects can increase the bioavailability of certain heavy metals to the sediment macrofauna (Bryan & Langston, 1992). Increases in organic loading can also influence macrofauna separately from the effects of heavy metals by increasing the abundances of small, tolerant, opportunistic species at high levels of loading while simultaneously decreasing the diversity and biomass of the overall community before the eventual population collapse (Pearson & Rosenberg, 1978). This means that simultaneous assessment of organic loading and sedimentary heavy metal content is essential to separate their effects and to identify possible interactions.

Sediment macrofaunal communities have long been used for environmental monitoring of marine habitats (Ryu et al., 2011; Villnäs, Hewitt, & Norkko, 2015). Various multi-metric benthic indices, which condense macrofaunal species abundance data into an easily understandable and comparable single number, have been developed to aid these monitoring efforts. In the Baltic Sea two ecological status indices, Benthic Quality Index (BQI; Leonardsson, Blomqvist, & Rosenberg, 2009) and Brackish water Benthic Index (BBI; Perus et al., 2007), have been developed or adapted for use and are being considered for application across the region (Villnäs et al., 2015). Earlier studies have also used Shannon-Wiener's Index (H') as a rough estimate of ecological status (Luotamo, 1974). It is a well-established species diversity index, and a

component in calculating BBI (Begon, Howarth, & Townsend, 2014; Villnäs et al., 2015). BQI and BBI have been proven to react to bottom-water oxygen, a known disturbance factor in the Baltic Sea, but are also sensitive to salinity and reach lower values in naturally low-salinity environments like the Gulf of Finland (Villnäs et al., 2015). The sensitivity to low salinities is also a feature of H', which makes its use in the naturally low-salinity northern Baltic Sea particularly difficult (Zettler, Schiedek, & Bobertz, 2007). Furthermore, BQI is known to be heavily influenced by the calculation of the species sensitivity values, which requires a large dataset from the region under study (Leonardsson et al., 2009). Of the two indices, BQI is known to be more sensitive to variation in the number of individuals, while BBI is influenced by species diversity, likely because the diversity index H' is included in its formula (Villnäs et al., 2012).

Several sampling techniques have been used for taking macrofaunal samples over the decades. An old and commonly used sampling technique is a van Veen grab, which was already in use in 1936 according to Elliott & Drake (1981) (Leonardsson et al., 2009; Rousi et al., 2013; Villnäs et al., 2015). It samples a large area of sediment, which is time consuming to process, but there are extensive time series of samples from locations like Tvärminne, where the current study was conducted, which makes it useful for ecological studies (Rousi et al., 2013). GEMAX twin corer is a more recently developed sediment sampling method but it has been adopted for macrofaunal sampling due to a smaller area sampled volume, which makes sample processing much faster (Kauppi, Norkko, & Norkko, 2018; Kauppi et al., 2017; Winterhalter, 2001). However, it is uncertain whether samples taken with GEMAX corer are directly comparable to samples taken with van Veen grab, which could have implications when comparing GEMAX studies to studies using the van Veen grab. Different sampling methods have been compared to each other in multiple past studies, but the results have been mixed. Souza & Barros (2014) found no significant differences between sampling methods when comparing a van Veen grab to a diver-operated corer, while Lampadariou, Karakassis, & Pearson (2005) reported significant differences in biomass and species diversity between these two methods. It should be noted, however, that the grab used by Souza & Barros was smaller than the one used in this study, which could affect the results if the community is spatially heterogenous.

The overall goal of this study is to investigate the potential influence of sediment heavy metal pollution on the macrofaunal community. By studying macrofaunal community composition along a transect outwards from the heavy metal pollution point source, it should be possible to assess the changes and succession in the community structure across the pollution gradient and compare them to possible changes in the other environmental factors. The study aims to answer the following research questions:

1. Does sediment heavy metal concentration affect macrofaunal community composition and how does it compare to the effects of other environmental parameters?
2. Is the possible effect of heavy metal pollution detected by the common macrofaunal community indices (BBI, BQI)?
3. Does the sediment sampling technique (i.e., van Veen grab versus GEMAX corer) have a noticeable effect on the macrofaunal results?

# 2. Materials & Methods

## 2.1 Study site and macrofaunal sampling

The study site was an area surrounding an old steel works factory located on the shoreline of the Baltic Sea. The steel works factory, active between 1961-2012, was located in the industrial area of Koverhar, east of Hanko on the shore of the Gulf of Finland. The factory was last operated by FNsteel Oy and included its own deep harbour (Jaakkonen, 2016). A previous study showed elevated sedimentary heavy metal concentrations near the steel works (Luotamo & Luotamo, 1979) and six locations, some of which had been used in the previous study, were chosen along the assumed pollution gradient as sampling stations for the current project (Table 1, Figure 1).

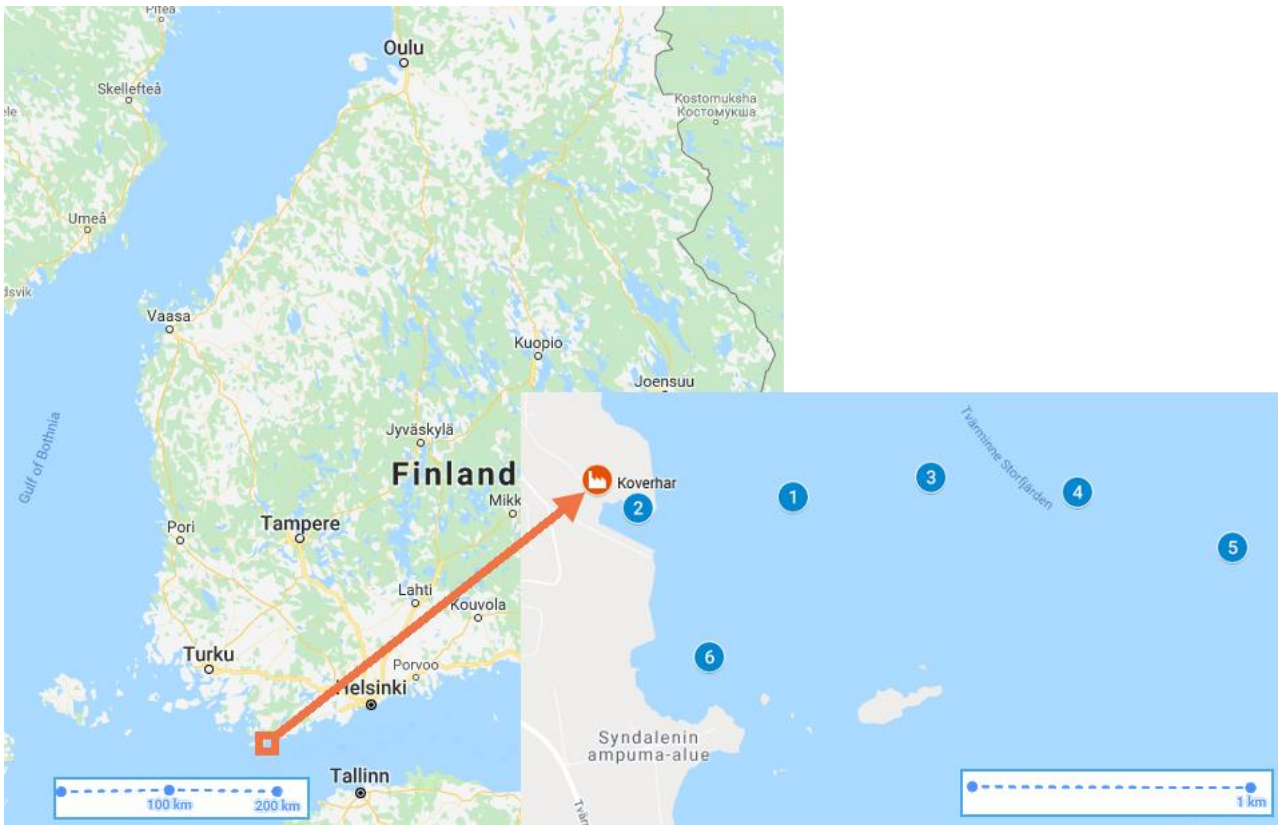


*Figure 1. The location of the site and sampling stations.*

| | Coordinates | |
|---|---|---|
| **station** | *N* | *E* |
| 1 | 59°52'48"N | 23°14'9.3"E |
| 2 | 59°52'46.56"N | 23°13'30.42"E |
| 3 | 59°52'50.4"N | 23°14'43.8"E |
| 4 | 59°52'48.48"N | 23°15'20.7"E |
| 5 | 59°52'41.52"N | 23°15'59.46"E |
| 6 | 59°52'27.78"N | 23°13'48.24"E |

*Table 1. Coordinates of the sampling stations (in DMS form).*

The complete transect was sampled in October and November of 2016. Stations 1, 2, 3, 4 and 5 form the main part of the transect, while station 6 represents a mouth of a former wastewater pipe coming from the industrial area. Some additional samples were collected from stations 1, 3, 5 and 6 in August 2017 to gain further insight into possible temporal changes in macrofaunal distribution and abundance.

Five macrofaunal sample replicates were taken at each station from the top 20 cm of the sediment with a GEMAX twin corer (sampling area 62,63 $cm^2$, 1,3 litres per sample

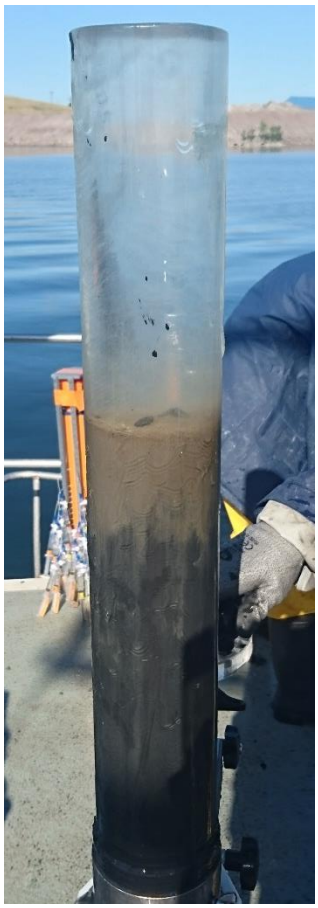

*Figure 2. GEMAX core sample in a sampling tube.*

when sampling to the depth of 20 cm, shown in Figure 2). Additionally, five van Veen grab macrofaunal samples (sampling area 1287 $cm^2$, maximum of 15 litres per sample, shown in Figure 3) were taken from stations 1 and 5 in 2016, and from station 1 in 2017, to compare the captured macrofaunal assemblages with the GEMAX samples.

The sediment macrofaunal samples were sieved, using 1,0 mm and 0,5 mm mesh sizes, into two size fractions. Both fractions were preserved in 70% ethanol, and when available, stained with rose bengal, to ease the identification of living specimens. The fractions were manually analysed, and the species were counted and identified to the lowest taxonomic level possible (genus or species depending on available resources). The abundances of individuals found in the macrofaunal samples were standardised to 1 $m^2$, hereafter referred to as standardised macrofaunal data. In addition, the relative abundances of different species (as percentages) were calculated for each replicate, hereafter referred to as relative abundance data. The individual shell lengths (to the closest 0,5 mm) and total sample wet weight of the bivalve *Limecola balthica* found in the 1 mm fraction were also measured from each sample, and the results standardised to 1 $m^2$. The measured lengths were divided into length classes and converted to relative abundances before analysis. For statistical analysis of the abundances the two fractions were combined, resulting in a sample equal to that acquired by using a 0,5 mm mesh only.

*Figure 3. van Veen sampling grab.*

Species diversity i.e. Shannon-Wiener's index (H') (calculated according to the formula in Perus et al. (2007)) and ecological quality indices Benthic Quality Index (BQI) (formula in Leonardsson et al. 2009)) and Brackish water Benthic Index (BBI) (formula in Perus et al. (2007)) were calculated, using the abundance data standardised to 0,1 $m^2$, to represent the species diversity and ecological status at each station using the following formulae:

$$H' = -\sum_{i=1}^{S} p_i \times \log p_i \qquad \log 2 - base\ used$$

where $S$ is the number of species or taxa in a sample and $p_i$ is the proportion of individuals belonging to the $i$th species.

$$BQI = \left[ \sum_{i=1}^{S_{classified}} \left( \frac{N_i}{N_{classified}} \times sensitivity\ value_i \right) \right] \times \log_{10}(S+1) \times \left( \frac{N_{tot}}{N_{tot}+5} \right)$$

where $S$ is the number of species or taxa in a sample, $S_{classified}$ is the number of taxa that have a sensitivity value, $N_i$ is the number of individuals in a taxon, $N_{classified}$ is the total number of individuals within taxa that have a sensitivity value, $sensitivity\ value_i$ is the sensitivity value for taxon $i$ and $N_{tot}$ is the total number of individuals in the sample (standardised to 0,1 $m^2$). The species sensitivity values used were the ones given in Appendix A of Leonardsson et al. (2009). Taxa not given a sensitivity value are excluded from the sensitivity factor but included in the total number of species and abundance factors.

$$BBI = \frac{\left[ \left( \frac{BQI}{BQI_{max}} \right) + \left( \frac{H'}{H'_{max}} \right) \right]}{2} \times \frac{\left[ \left( 1 - \frac{1}{AB_{tot}} \right) + \left( 1 - \frac{1}{S} \right) \right]}{2}$$

where $BQI_{max}$ is the maximum BQI value recorded within type of environment, $H'_{max}$ is the maximum H' value recorded within type of environment, $AB_{tot}$ is the total number of individuals in the sample (standardised to 0,1 $m^2$) (equal to $N_{tot}$ in BQI formula) and $S$ is the number of species or taxa in a sample. Maximum BQI and H' values used were the ones given for Ls 10+m in Table 6 of Vuori et al. (2009).

The boundary values between ecological classification categories were calculated to serve as rough estimates for the status of diversity and the environment for the purposes of this thesis (Table 2). For H' the boundary values were based on the values suggested for southwestern inner archipelago areas deeper than 10 meters (abbreviated Ls 10+m in Perus et al. (2007)). The values were estimated by calculating the minimum value of the higher category and maximum value of the lower category, which resulted in a range where the two categories overlap. The position of the boundary line was then set halfway within this range.

For BQI the positions of the boundaries were based on the boundaries suggested in Figure 5 of Leonardsson et al. (2009), which were calculated for Krabbfjärden on the Swedish eastern coast, and thus might not be entirely representative for the Koverhar study area. The only exception was the high-good boundary, which was set at 2/3 between the good-moderate boundary and the maximum value recorded for the Ls 10+m category in Vuori et al. (2009), as recommended by Leonardsson et al. (2009).

The BBI boundary values were based on the current Finnish national standards for coastal waters in category Ls 10+m as presented in Vuori et al. (2009) Appendix 3.3.

| | H' | BQI | BBI |
|---|---|---|---|
| high / good | 2,03 | 8,99 | 0,56 |
| good / moderate | 1,69 | 4,00 | 0,34 |
| moderate / poor | 1,10 | 2,67 | 0,22 |
| poor / bad | 0,50 | 1,33 | 0,11 |

*Table 2. The boundary values of ecological status for the indices.*

## 2.2 Environmental background data

The environmental and heavy metal background data was collected outside the scope of this thesis and is used here only to provide information on the prevailing environmental conditions at the study site. A description of sampling, overview of the data and the selection of variables for further analysis is presented in Appendix 1: Environmental conditions.

For comparisons with literature and between stations, sediment metal concentrations (units mg/kg or µg/g) were transformed into concentrations in standard sediment (sediment organic matter dry weight 10% and clay dry weight 25%), using the following formula from Suomen ympäristöministeriö (2004):

$$Ckorj. = C * \frac{(a + b * 25 + c * 10)}{(a + b * clay + c * organic\ matter)}$$

where Ckorj. = metal concentration in standard sediment (dry matter, units mg/kg or µg/g), C = measured metal concentration (dry matter, units mg/kg or µg/g), clay = measured proportion of clay (as percentages of dry weight), organic matter = measured proportion of organic matter (as percentages of dry weight) (maximum value of 30, higher values are inserted as 30) and a, b and c are constants given for each metal in the table in Appendix 1 of Suomen ympäristöministeriö (2004). This allowed the measured concentrations to be compared to the normal background concentrations (Kemppainen, 2000) and high (potentially toxic) concentrations (Suomen ympäristöministeriö, 2004).

Pollution Load Index (PLI) was calculated, based on the standardised metal concentrations (see 2.2), to represent heavy metal pollution at each sampling station using the following formula from Tomlinson et al. (1980):

$$PLI = \sqrt[n]{CF_1 \times CF_2 \times \ \ldots \times CF_n}$$

where $CF_{metal} = \ CH_{measured} \ / \ CH_{background}$, where $CH_{measured}$ is the concentration of heavy metal measured in the sediment sample and $CH_{background}$ is the background concentration of the heavy metal in the region. The background concentrations used for the calculations were taken from Table 2 in Kemppainen (2000).

## 2.3 Data analysis

All statistical analyses and graphs were done with R (R Core Team, 2017), using RStudio (RStudio Team, 2016) and the packages dplyr (Wickham et al., 2017), readxl (Wickham & Bryan, 2017), writexl (Ooms, 2017), reshape2 (Wickham, 2007), scales (Wickham, 2017), ggplot2 (Wickham, 2009), ggrepel (Slowikowski, 2018), rcompanion (Mangiafico, 2018), car (Fox & Weisberg, 2011), vegan (Oksanen et al., 2018) and pairwiseAdonis (Martinez Arbizu, 2017). The standardisation of macrofaunal samples, and the calculation of macrofaunal indices BQI and BBI and pollution load index PLI were done with Microsoft Excel. The calculation of H' was done with R (vegan). All index calculations were done from abundance data standardised to 0,1 $m^2$ as per instructions in the original publications.

The similarities and differences in the macrofaunal community composition between stations were compared to each other with non-metric multidimensional scaling (nMDS) ordinations. The ordinations were based on station Bray-Curtis distance matrix calculations done with relative abundance data. The environmental data distance matrix was calculated using the maximum distance method, and the hierarchical cluster analysis using the average (UPGMA) method.

The significance of these comparisons was confirmed with PERMANOVA (vegan) using the GEMAX macrofaunal community data with relative abundances, as the focus was on species composition, not on absolute abundances. Both the standardised abundance data and the relative abundance data were tested in the GEMAX vs. van Veen comparisons to see if the additional information could reveal differences that were otherwise not apparent. As the data was mainly not normally distributed, a non-parametric Kruskal-Wallis test was used to test the differences in the total abundances and index values between stations. Pairwise PERMANOVA (pairwiseAdonis) and pairwise Mann-Whitney U tests with Benjamini & Hochberg corrections were used as post-hoc tests for PERMANOVA and Kruskal-Wallis, respectively. Spearman's rank correlation was used to test for correlations between index values and when comparing index values to environmental variables. In a few cases, where the data was normally distributed and there was no need to make comparisons with Kruskal-Wallis tests, a t-test (base R) was used.

A principal component analysis (PCA, base R) was used to identify correlating environmental variables so that non-correlating variables could be selected (Appendix Figure 3). The selected environmental variables (PLI (Pollution Load Index representing heavy metals), NH4_inv_PW (pore water $NH_4$ inventory), season_year (sampling season and year) and C_1cm (sediment organic carbon content in the top 1 cm)) were then compared to the macrofaunal community with canonical correspondence analysis (CCA, vegan) to examine the influence of environmental variables on macrofaunal community composition. Of the environmental variables measured, PLI correlated positively with bottom-water oxygen concentration, sediment grain size and sediment C/N ratio, season_year with proportion of 13C in the top 1 cm, bottom-water temperature and salinity and C_1cm with water depth, sediment clay content, pore water $H_2S$ inventory, while NH4_inv_PW did not positively correlate with any other variable.

# 3. Results

## 3.1 Macrofaunal data and species distribution in GEMAX samples

A total of 24 species were encountered in the GEMAX macrofaunal samples, with a median of 6 species per replicate and a median of 8,5 species per station. The highest number of species (11) was found at stations 2 in autumn 2016 and 6 in summer 2017, while the lowest number (6) was found at station 3 in summer 2017. The full standardised dataset is presented in Appendix 2: Macrofaunal data.

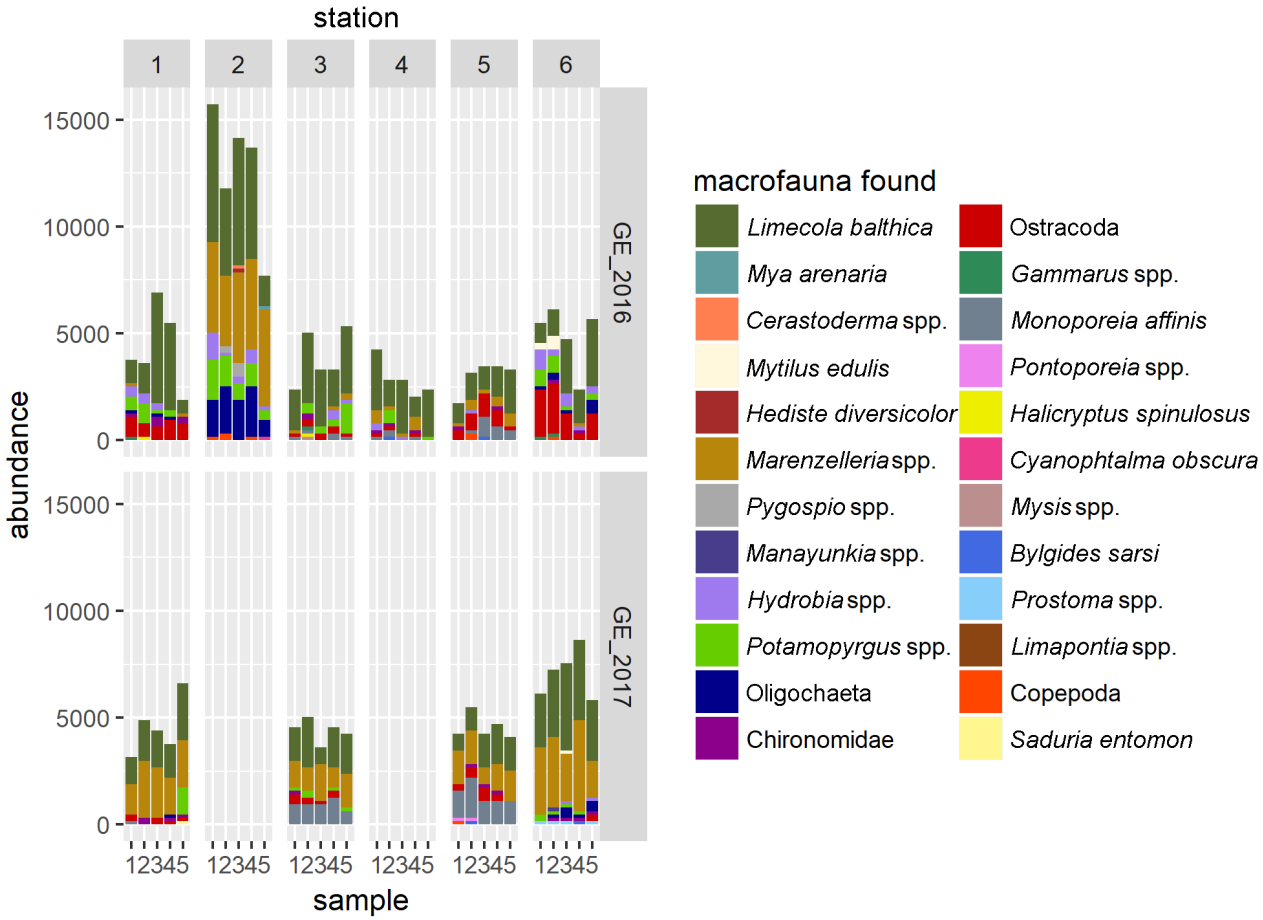


*Figure 4. List of the species found in GEMAX samples, and their abundances (per m2) sorted by year and station GE = GEMAX.*

The overall mean density of macrofauna was 5215 individuals/m$^2$. In 2016, the highest macrofaunal densities (with replicate median of 13675 ind./m$^2$) were found at station 2 (Figure 4), although the variation between replicates was relatively large (interquartile range 2358). Relatively high density was also recoded at station 6 (median of 5501 ind./m$^2$) and in some replicates from stations 1 and 3. However, in the case of stations 1 and 3 there was notable variation between replicates (medians of 3772 ind./m$^2$ and 3301 ind./m$^2$, interquartile ranges of 1886 and 1729, respectively). The densities of macrofauna were somewhat lower at stations 4 and 5 (medians of 2829 ind./m$^2$ and 3301 ind./m$^2$, respectively) and the variation between the replicates was more even (interquartile ranges of 472 and 314, respectively). In terms of density of individuals station 2 was the only significantly different station within the GEMAX autumn 2016 subgroup (pairwise Mann-Whitney U test comparisons with other stations, $p < 0,05$).

In summer 2017 the highest macrofaunal densities were recorded in GEMAX samples from station 6 (median of 7230 ind./m$^2$, interquartile range of 1415), which was also significantly different from stations 3 and 5 (pairwise Mann-Whitney U test, $p < 0,05$ when comparing station 6 to stations 3 and 5, $p = 0,063$ when comparing station 6 to station 1) in terms of density of individuals. Stations 1 (median of 4401 ind./m$^2$, interquartile range of 1100) and 6 had higher variation in macrofaunal abundances between the replicates, while station 3 (median of 4558 ind./m$^2$, interquartile range of 314) and station 5 (median of 4244 ind./m$^2$, interquartile range of 472) replicates were more similar to each other.

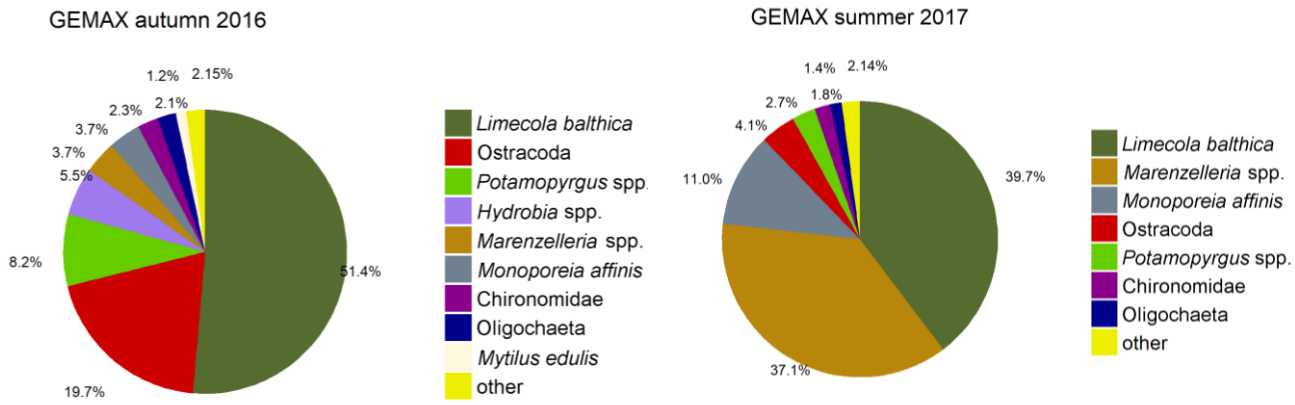


*Figure 5. Proportions of species found in GEMAX samples from different seasons and years (stations 1, 3, 5 and 6 only). Species with abundances less than 1% of the total abundance were sorted into the category "other".*

In all GEMAX samples the most common species was the bivalve *Limecola balthica*, making up 51,4 % of counted individuals in 2016 and 39,7 % of individuals in 2017 (Figure 5). It was present in all samples, although its densities varied from 419,6 individuals / m$^2$ in autumn 2016 at station 5 to 6444,5 individuals / m$^2$ in autumn 2016 at station 2 (Figure 4). Polychaeta *Marenzelleria* spp. showed a strong seasonal trend, being almost completely absent in autumn 2016 (3,7 % of individuals when only counting the stations that had samples taken in both years) but representing 37,1 % of individuals in summer 2017. Station 2 (the station closest to the factory) in autumn 2016 GEMAX samples (shown in Figure 4, excluded from Figure 5) was unique, as it accounted for 31,1% of all *Limecola balthica*, 81,9% of all *Marenzelleria* spp. and 83,1% of all Oligochaeta within the autumn 2016 subgroup, while having no Ostracoda at all.

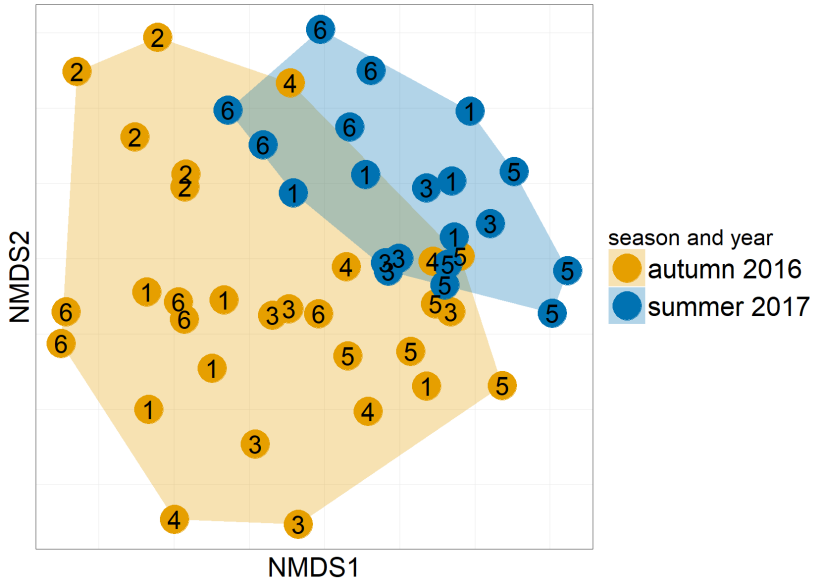


*Figure 6. nMDS plot based on macrofaunal relative abundances from GEMAX samples. Numbers represent stations.*

Non-metric multidimensional scaling analysis (nMDS) was used to get an overview of the similarities of macrofaunal communities between stations. Samples taken in autumn 2016 formed a separate cluster to samples taken in summer 2017 (Figure 6), implying that the samples from the summer 2017 were clearly different from those taken in autumn 2016. Most replicates clustered close together, showing that the samples from the same station were relatively similar to one another, although exceptions were also evident (e.g. autumn 2016 station 4).

| autumn 2016 | | | | | | |
|---|---|---|---|---|---|---|
| station | | 2 | 3 | 4 | 5 | 6 |
| 1 | R2 | 0,558 | 0,261 | 0,243 | 0,253 | 0,051 |
| | p | 0,026 | 0,080 | 0,068 | 0,080 | 0,709 |
| 2 | R2 | | 0,722 | 0,519 | 0,663 | 0,591 |
| | p | - | 0,026 | 0,026 | 0,026 | 0,026 |
| 3 | R2 | | | 0,061 | 0,469 | 0,406 |
| | p | - | - | 0,709 | 0,026 | 0,026 |
| 4 | R2 | | | | 0,310 | 0,397 |
| | p | - | - | - | 0,056 | 0,026 |
| 5 | R2 | | | | | 0,346 |
| | p | - | - | - | - | 0,052 |
| **summer 2017** | | | | | | |
| 1 | R2 | | 0,487 | | 0,626 | 0,230 |
| | p | - | 0,018 | - | 0,018 | 0,106 |
| 3 | R2 | | | | 0,167 | 0,521 |
| | p | - | - | - | 0,223 | 0,018 |
| 5 | R2 | | | | | 0,679 |
| | p | - | - | - | - | 0,018 |

*Table 3. Significant differences in macrofaunal community composition between stations calculated with PERMANOVA based on relative abundance data. Green background highlights a significant difference.*

The macrofaunal communities from the stations were compared to each other using PERMANOVA. Each year was considered separately as the nMDS ordination (Figure 6) suggested there were noticeable differences between years. When comparing the stations using relative abundance data, significant differences were found between stations within both years (autumn 2016 R2 = 0,543, Pr(>F) = 0,001 and summer 2017 R2 = 0,611, Pr(>F) = 0,001). According to the post-hoc pairwise PERMANOVA (pairwiseAdonis) comparisons (Table 3), in autumn 2016 station 2 was significantly different from all other stations, station 3 was significantly different from stations 5 and 6, and station 4 was significantly different from station 6. In summer 2017 stations 1 and 6 were significantly different from stations 3 and 5.

The measured *Limecola* lengths and weights were also tested for differences between stations (Figure 7, Figure 8). In autumn 2016 the lengths ranged from 1,5 to 22,0 mm, with the median length at 4,5 mm, and the individual average wet weights from 0 to 1,39 g with the median of 0,26 g. In summer 2017 the lengths ranged from 1,5 to 20,0 mm with the median of 4,0 mm and the weights from 0,01 g to 0,61 g with a median of 0,10 g. In PERMANOVA tests no significant differences in length distribution between stations were detected in autumn 2016 (R2 = 0,205, Pr(>F) = 0,12). Summer 2017 seemed to have some significant differences between stations in the length distributions (R2 = 0,245, Pr(>F) = 0,02), but the differences were not confirmed by the post-hoc test. No significant differences were detected in the *Limecola* wet weights from autumn 2016 (Kruskal-Wallis chi-squared = 5,470, df = 5, p-value = 0,361), but summer 2017 weights were significantly different from each other (Kruskal-Wallis chi-squared = 9,034, df = 3, p-value = 0,029). Post-hoc Mann-Whitney U test revealed that station 5 average wet weights were significantly different from both station 1 and station 6 (p-value = 0,048 in both cases).

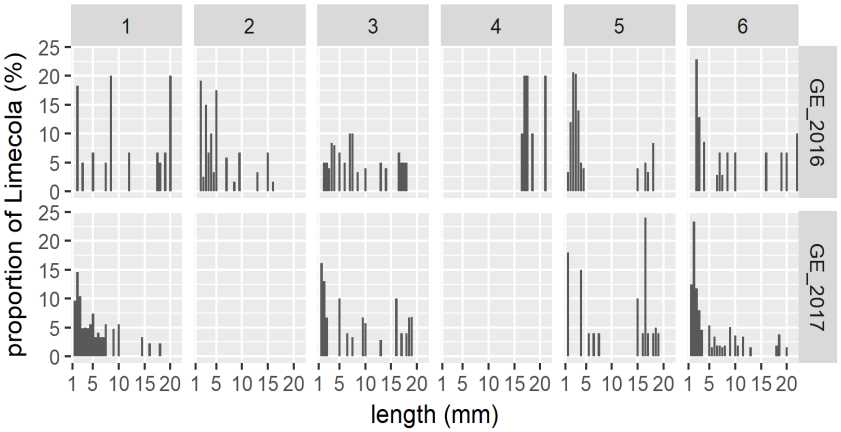


*Figure 7. The relative distribution of Limecola balthica in length classes within station in 2016 and 2017. GE = GEMAX.*

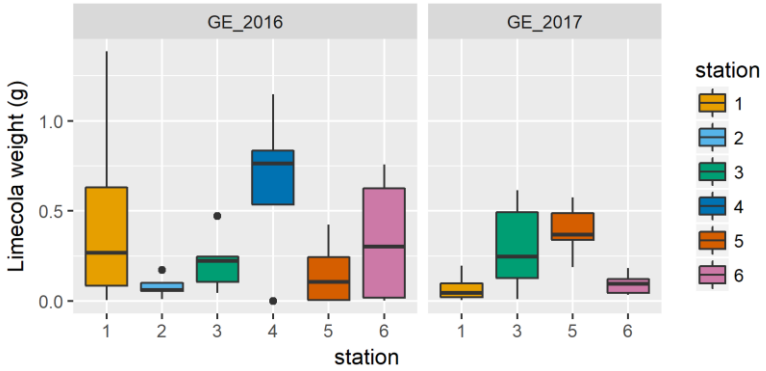


*Figure 8. Distribution of Limecola balthica average wet weights (per m2). GE = GEMAX.*

## 3.2 Influence of heavy metals and other environmental factors on macrofaunal community

Canonical correspondence analysis (CCA) based on the macrofaunal abundances and environmental background data had four constrained axes, the first three of them significant, and accounted for 32% of the variation in macrofaunal community composition (Figure 9). The first CCA-axis accounted for 52,41% of the variation in the model and seemed to be connected to the seasonal variation and pore water $NH_4$, with seasonal variation as the stronger influence. The second axis accounted for 33,58% of the model variation and was connected to the sediment properties associated with PLI with the organic carbon having a slightly smaller effect. All chosen variables significantly influenced the macrofaunal community composition. PLI, NH4_inv_PW and season_year were all highly significant (Pr(>F) = 0,001), while C_1cm was significant (Pr(>F) = 0,002).

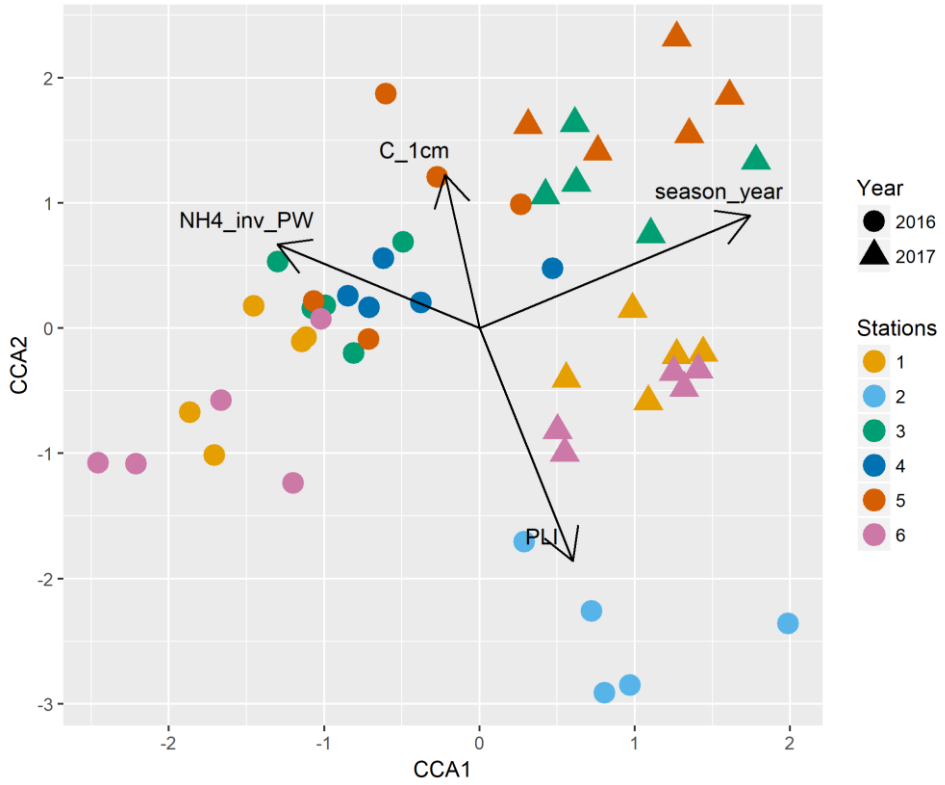


*Figure 9. Canonical correspondence analysis (CCA) of the relative macrofaunal abundances, NH4 pore water inventory (NH4_inv_PW), organic carbon content of the top 1 cm (C_1cm), pollution load index (PLI) and the time of sampling (season_year).*

## 3.3 Species diversity and ecological status indices as indicators of heavy metal pollution

The three indices used for estimating the diversity and ecological status of the environment were Shannon-Wiener's index (H'), BQI and BBI (Figure 10).

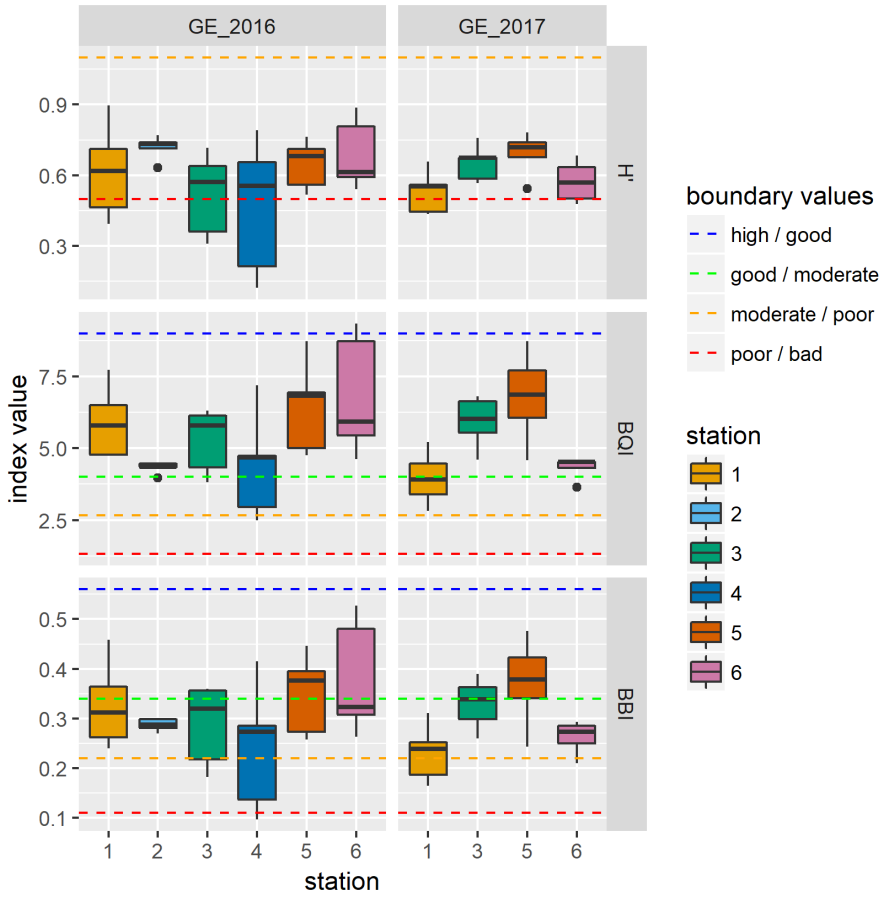


*Figure 10. Macrofaunal diversity and ecological status indices based on GEMAX macrofaunal data standardised to 0,1 m2 as required in the index formulae. The horizontal lines show the boundaries between environmental classification categories. The boundary values for H' were calculated from Perus et al. (2007) and BQI boundaries from Leonardsson et al. (2009). The BBI boundary values are from current Finnish national standards (Vuori et al., 2009).*

The median H' index values ranged from 0,55 (summer 2017 station 1) to 0,73 (autumn 2016 station 2), with the overall median value of 0,62. H' showed no differences between stations in either year and estimated the environmental status to be "poor" on all studied stations on both years. On stations 1, 3 and 4 some replicate samples even indicated "bad" diversity status in both years.

The BQI index ranged from 3,90 (summer 2017 station 1) to 6,87 (summer 2017 station 5) with a median value of 4,76. Clear differences were seen in the BQI index between different stations. In samples from autumn 2016 station 2 was significantly different from stations 1, 5 and 6 (pairwise Mann-Whitney U, p-value 0,04 in all cases). In summer 2017 stations 1 and 6 were significantly different from stations 3 and 5 (pairwise Mann-Whitney U, p-value 0,024 in all cases). BQI indicated the environmental status to be "good"

on most stations, excluding station 1 in 2017 and some replicate samples from 2016 from station 4, which classified as "moderate" status. BQI was significantly correlated with H' (rho = 0,700, p-value < 0,001).

The BBI ranged from 0,24 (summer 2017 station 1) to 0,38 (summer 2017 station 5) with a median value of 0,30. In contrast to BQI, the BBI seemed to obscure some of the differences between the stations and no significant differences were seen among the samples taken in autumn 2016. In summer 2017, Kruskal-Wallis test indicated significant differences in BBI between stations, but the post-hoc pairwise Mann-Whitney U test failed to find them. The differences closest to being significant were station 1 compared to stations 3 and 5 (pairwise Mann-Whitney U, p-value 0,095 in both cases) and station 6 compared to station 3 (pairwise Mann-Whitney U, p-value 0.111) and station 5 (pairwise Mann-Whitney U, p-value 0.143). BBI estimated the environmental status to be "moderate" on stations 1, 2, 3, 4 and 6 and "good" on station 5 in both years. However, some replicate samples from stations 1 (in 2016), 3 (both years) and 6 (in 2016) also reached "good" status and some from stations 1 (in 2017), 3 (in 2016), 4 (in 2016) and 6 (in 2017) classified as "poor". BBI was significantly correlated with both H' (rho = 0,888, p-value < 2,2e-16) and BQI (rho = 0,924, p-value < 2,2e-16).

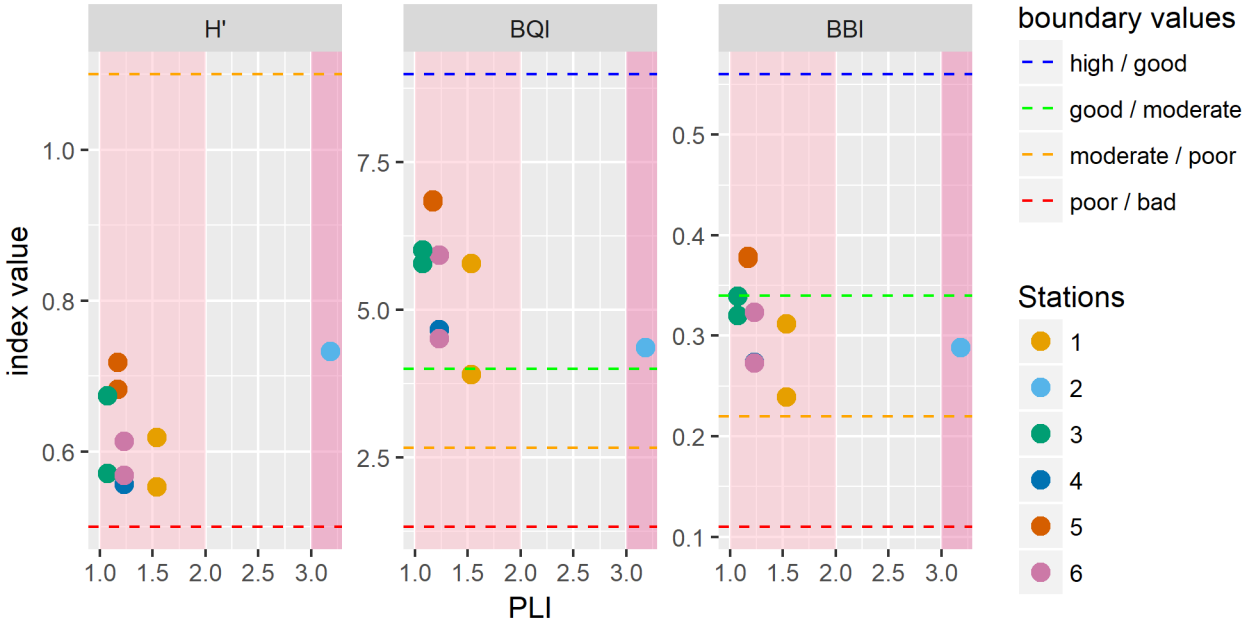


*Figure 11. Station median values of the macrofaunal diversity and ecological status indices from GEMAX samples (calculated per 0,1 m2) plotted against the pollution load index (PLI). PLI values 1-2 indicate moderate pollution (light pink shading), 2-3 heavy pollution (no shading) and >3 extremely heavy pollution (dark pink shading). The legend of boundary values refers to the boundaries of species diversity and ecological status indices.*

The macrofaunal and ecological status indices (H', BQI and BBI) were compared to PLI to see if they reflected the increasing pollution load in the sediments (Figure 11). No correlation was detected between H' and PLI (rho = 0,001, P-value = 0,996). However, BQI had a significant negative correlation with PLI (rho = -0.394, p-value = 0,005) and BBI, while not significantly correlated, also came very close (rho = -0,247, p-value = 0,083). Based on our samples the species diversity H' did not reflect the detected levels of heavy metal pollution, while the ecological status indices BQI and BBI do detected some deterioration of status in connection with higher values of PLI. Neither bottom-water oxygen concentration nor salinity was significantly correlated with any of the macrofaunal indices, and of the variables included in the CCA the only significant correlation was between BQI and pore water $NH_4$ concentration (rho = 0,411, p-value = 0,003).

## 3.4 Comparison of macrofaunal sampling methods: GEMAX cores versus van Veen grabs

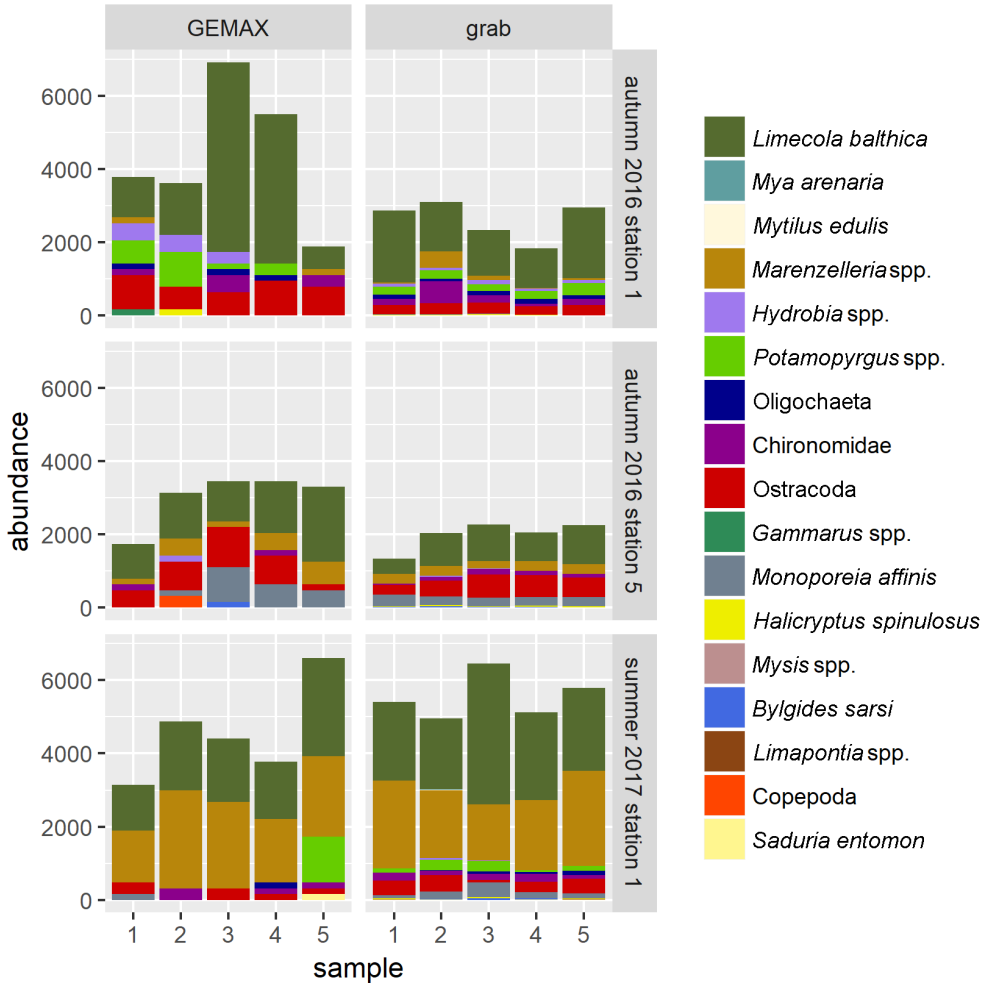


*Figure 12. List of species and their abundances (per m2) from autumn 2016 stations 1 and 5, and summer 2017 station 1 from GEMAX and van Veen grab sampling methods.*

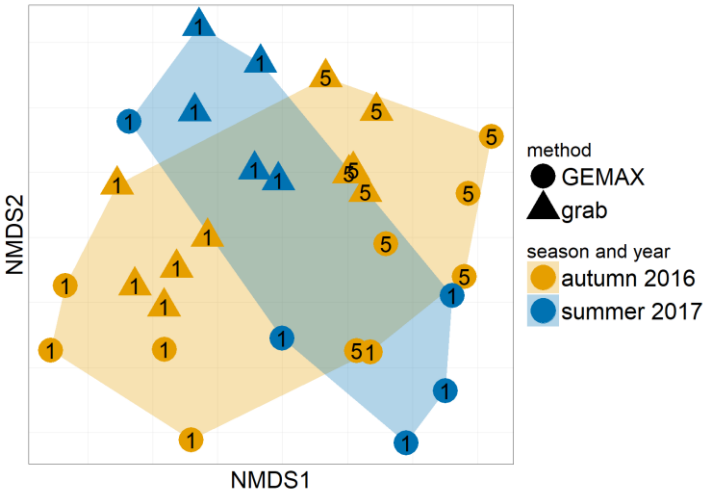


*Figure 13. nMDS plot based on macrofaunal abundance percentages from GEMAX and van Veen grab samples shows the similarity of the samples to each other.*

To make the comparison between the two methods, only the data from station 1 (autumn 2016, summer 2017) and 5 (autumn 2016 only), from which both GEMAX and van Veen grab samples were taken, was analysed (Figure 10). The mean individual density was 3971 individuals / $m^2$ for GEMAX samples, while the van Veen grab samples had a mean individual density of 3383 ind./$m^2$. There was no statistically significant difference in individual density between methods (Mann-Whitney U test, W = 146, p-value = 0,171).

There were slight differences in species distribution between methods. In autumn 2016 the van Veen grab samples appeared to have slightly more *Marenzelleria* spp.,

Oligochaeta and Chironomidae than the GEMAX samples (Figure 12). The GEMAX-grab nMDS (Figure 13) shows no clear distinction between years. However, the samples taken with different methods appeared more similar to each other in autumn 2016, while in summer 2017 the nMDS indicated a more noticeable difference between the sampling methods as the GEMAX and grab samples were clearly spatially separated (Figure 13). Statistically there was no significant difference between sampling methods when testing the relative abundance data with PERMANOVA (Pr(>F) = 0,533). However, when the PERMANOVA comparison was done with abundance data standardised to 1 $m^2$ and tested separately for each station, a significant difference in species distributions from different sampling methods was seen at stations 1 (Pr(>F) = 0,014) and 5 (Pr(>F) = 0,032) in autumn 2016, but not at station 1 (Pr(>F) = 0,227) in summer 2017.

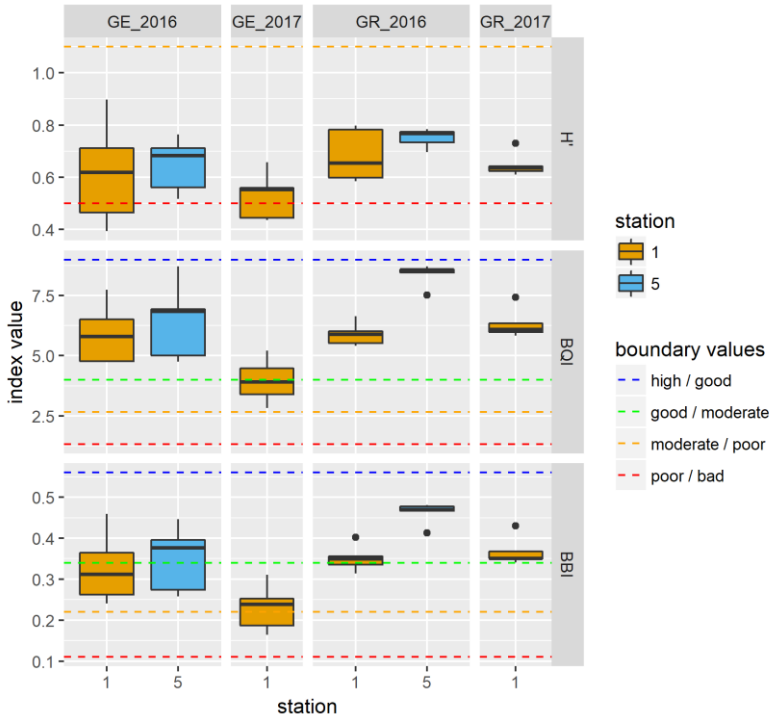


*Figure 14. Macrofaunal diversity and ecological status indices based on GEMAX and van Veen grab macrofaunal data standardised to 0,1 m2. GE = GEMAX, GR = van Veen grab. The horizontal lines show the boundaries between environmental classification categories. The boundary values are the same as in Figure 6.*

Clear differences were observed in the indices calculated with the different methods (Figure 14, Figure 15): H' (Mann- Whitney U test, W = 58, one-way p-value = 0,012), BQI (Mann- Whitney U test, W = 54, one-way p-value = 0,007) and BBI (Mann- Whitney U test, W = 45, one-way p-value = 0,002). In general, it seemed that GEMAX samples estimated lower index values (lower diversity or lower ecological status) and had more scatter between the replicate samples than the van Veen grab samples. Further testing showed that in the case of H' the differences were not significant when methods were compared within each station separately, although they came close on both autumn 2016 station 5 (W = 3, p-value = 0,056) and summer 2017 station 1 (W = 4, p-value = 0,095). In the case of BQI a significant difference was detected in summer 2017 station 1 (W = 0, p-value = 0,008), while for BBI there was a significant difference both in autumn 2016 station 5 (W = 1, p-value = 0,016) and summer 2017 station 1 (W = 0, p-value = 0,008).

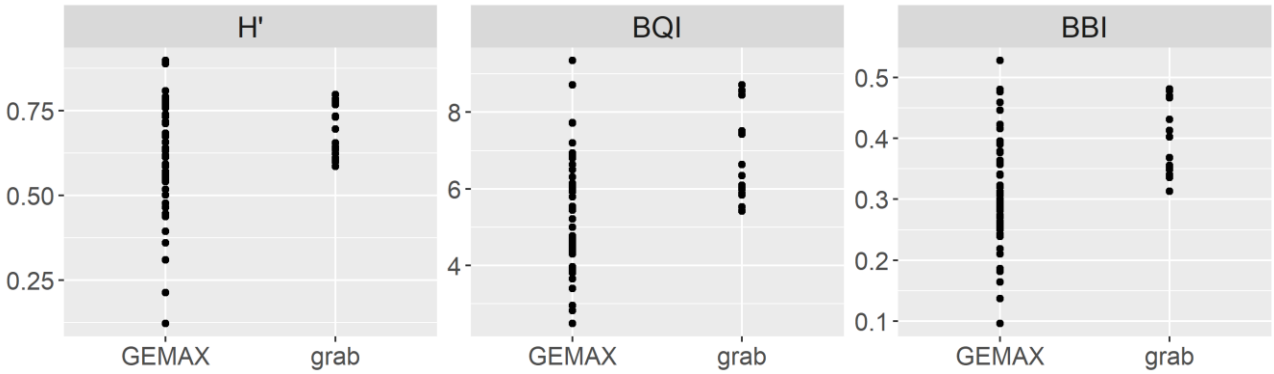


*Figure 15. Shannon-Wiener (H'), Benthic Quality Index (BQI) and Brackish water Benthic Index (BBI) values from stations 1 and 5 in autumn 2016 and station 1 in summer 2017 sorted by sampling method (grab = van Veen grab).*

# 4. Discussion

## 4.1 Influence of heavy metals on macrofauna and the strength of their effect compared to other environmental factors

### 4.1.1 A case for the effects of heavy metal pollution: station 2

Substantial heavy metal pollution was found in the immediate vicinity of the factory area (Figure 11). The PLI value for station 2 showed extremely high heavy metal pollution according to the PLI reference values given in Bastami et al. (2017), with Cr, Cu, Ni and Zn exceeding the limits of potentially harmful concentrations as defined by Suomen ympäristöministeriö (2004) (Figure 11, Appendix Figure 1, Appendix Table 1). The rest of the stations all ranked as moderately polluted, with station 1 slightly more polluted than the others. The most polluted sediments were found at the depth of 4-8 cm (Appendix 1: Environmental conditions). This differs from the observations made by Luotamo & Luotamo (1979), who in the 70's identified the levels of Cd, Pb and Zn as the most elevated, and measured extremely heavy pollution on stations 2 and 6, heavy pollution on station 3 and moderate pollution on stations 4 and 5, with station 1 hovering on the edge of the heavily polluted area, with the highest levels of pollution at the topmost sediment layers. However, the metal pollution classification index and criteria used by Luotamo & Luotamo (1979) to identify heavy and moderate pollution were different, so the results are not directly comparable.

Station 2 also stood out from all other stations in autumn 2016 in terms of environmental variables and macrofauna (Figure 4, Table 3, Appendix Table 1). It had nearly three times the abundance of individuals found on other stations, possibly as a result of the larger grain size, as individual abundances tend to be higher on coarser sediments with lower organic matter content (Gammal et al., 2019). The station was heavily populated by species tolerant of environmental stress, most notably the bivalve *Limecola balthica*, the polychaeta *Marenzelleria* spp. and the annelid subclass Oligochaeta (Figure 4) (Leonardsson et al., 2009). It was also notably lacking in the environmentally sensitive crustacean Ostracoda common on other stations in autumn 2016, further indicating that the environmental conditions of station 2 were likely stressful to macrofauna (Leonardsson et al., 2009). The large proportion of *Marenzelleria* spp. encountered was unexpected, as the genus tends to prefer deeper sites with more stable environmental conditions during late autumn and winter, and is usually absent from shallower, low organic matter stations during this time (Kauppi et al., 2018). In our data, their behaviour was almost completely opposite (Figure 4). This behaviour of *Marenzelleria* spp. could have something to do with the three different species in the genus in the Baltic Sea region (*M. viridis*, *M. neglecta* and *M. arctia*), each with slightly different population dynamics that still are not fully understood due to the genus only having arrived in the Baltic Sea region in the 1980's (Kauppi et al., 2018). Perhaps future research will shed more light on the matter. Due to its unusually large grain size, station 2 also had a few stray individuals from a number of species that prefer sandy sites and were not observed on other sampling stations (*Mya arenaria*, *Cerastoderma* spp., *Hediste diversicolor*, *Pygospio* spp. and *Cyanophtalma obscura*) (Gammal et al., 2019). These species are also known to be tolerant of environmental stress, so they might have an advantage over less tolerant species such as *Halicryptus spinulosus*, *Microstomum lineare, Manayunkia aestuarina*, *Limapontia capitata*, *Theodoxus fluviatilis* (although several dead shells were encountered) and Nemertea, that have been observed in similarly sandy sites but were absent from station 2 (Kauppi et al. (2017); Leonardsson et al. (2009)).

Compared to the reports of Luotamo & Luotamo (1974, 1977, 1979), the modern day station 2 had a noticeable lack of environmentally sensitive crustacean *Monoporeia affinis* and a reduction in the proportion of the polychaete *Hediste diversicolor*, both species found in abundance in the 70's (Leonardsson et al., 2009). Even back then, though, *M. affinis* exhibited great variation on this station between sampling years. They used to be abundant in the 1960's, but have since been on the decline due to a combination of environmental pressures (mainly from changes in temperature and salinity and possibly eutrophication) and possible interspecies competition with *L. balthica* and *Marenzelleria* spp. (Rousi et al., 2013). The abundance of *L. balthica* also varied rather dramatically between the years in the 70's, but as station 2 was sampled only on one year in this study, it is not possible to say whether the drastic differences between years were still occurring. Strangely, Oligochaeta, *Hydrobia* spp., *Potamopyrgus* spp. and *Pygospio* spp. were also missing from the samples taken from station 2 in the 70's. It should be noted, that the Luotamo samples were sieved with a slightly larger mesh size (0,6 mm) and the samples were taken in September, which falls between our sampling months (Luotamo, 1974). Both of these could potentially contribute to the observed macrofaunal differences. It is also possible that the long-term changes in the Baltic Sea region detailed in Rousi et al. (2013) could have affected the abundances of these species.

### 4.1.2 The influence of other environmental variables: the stations with similar PLI in autumn 2016

The significant differences seen in the macrofaunal data between autumn 2016 station 3 and stations 5 and 6, and between stations 4 and 6, seemed also related to environmental stress (Table 3). While stations 3 and 4 were dominated mostly by the tolerant *L. balthica*, making up roughly 2/3 of the individuals, stations 5 and 6 had a more varied dominance of *L. balthica* and the more sensitive Ostracoda, and in the case of station 5 also a presence of *Marenzelleria* spp. and the highly sensitive *Monoporeia affinis* (Figure 4) (Leonardsson et al., 2009). This seemed to indicate that the conditions were more stressful on stations 3 and 4. The CCA supported the similarity of environmental influences on stations 3 and 4, as they were clustered close together (Figure 9). Some replicates of station 5 also clustered near them, but most of them were more influenced by the high sedimentary organic carbon in the station (Figure 9). Station 6 had the very little overlap with the other three stations, probably due to its shallowness, larger grain size and high pore water $NH_4$ (Figure 1, Figure 9, Appendix Table 1). However, looking at the environmental data, stations 3 and 4 both had four times higher concentration of pore water $H_2S$ than stations 5 and 6 (Appendix Table 1). $H_2S$ is a by-product of anaerobic decomposition, which seems to indicate a lack of oxygen in the sediment, and a toxic substance to sensitive macrofauna (Dunnette, Chynoweth, & Mancy, 1985). The bottom-water oxygen concentrations did not seem much lower on stations 3 and 4, although this may not be true in the sediment. Station 3 had a higher clay content and lower grain size of the sediment, which could explain this, but similar properties were not observed in the sediment of station 4 (Appendix Table 1). Comparing this to reports of Luotamo & Luotamo (1974, 1977, 1979) there was, on stations 3 and 6, the expected reduction in the proportion of *M. affinis* and a concurrent rise in the proportion of *M. balthica* since the 70's. Ostracoda, *Potamopyrgus* spp. and *Marenzelleria* spp. were new arrivals to station 3, while *Bylgides sarsi*, which made up 4% of the individuals found in 1977, was absent in 2016 and 2017. However, these changes are more likely to reflect general changes in the Baltic Sea rather than any shifts in sediment heavy metal concentrations (Rousi et al., 2013).

In terms of macrofauna, station 1 was significantly different only from station 2 within the autumn 2016 subgroup. *L. balthica* and Ostracoda dominated the station, with other species making up perhaps $1/5^{th}$ of the individuals. This made it something of a halfway point between stations 3, 4, 5 and 6, so that while they had differences amongst themselves, none of them were significantly different from station 1. Compared

to the reports of Luotamo & Luotamo (1974, 1977, 1979), the overall theme of fewer *M. affinis*, higher abundance of *L. balthica*, and the appearance of *Marenzelleria* spp., *Hydrobia* spp., *Potamopyrgus* spp. and Ostracoda in the modern station 1 samples holds true. In the CCA station 1 seemed to overlap with station 6 but was separated from the rest, which makes sense considering that the two stations were equally close to the shore and thus environmentally quite similar (Figure 1, Figure 9). Station 1 resembled station 6 in terms of depth and grain size but was more like station 3 in organic matter content (Appendix Table 1). C/N ratio on station 1 was slightly higher than on stations 3, 4, 5 and 6, but not as high as on station 2, probably reflecting the vicinity of the shore (Appendix Table 1). Pore water $NH_4$ on station 1 was higher than on any other station, pore water $H_2S$ was among the lowest measured, bottom water $O_2$ resembled that of station 5 and PLI there was second-highest after station 2, as expected (Appendix Table 1). These similarities seem to confirm that station 1 had some resemblance to several other stations but was not a precise match to any of them.

### 4.1.3 Other observations: the samples from summer 2017 and the *L. balthica* measurements

In summer 2017 the stations were clearly split into two groups, one with stations 1 and 6, and the other with stations 3 and 5, both environmentally and in terms of macrofauna. In macrofauna, the main difference between stations was that 1 and 6 were dominated equally by *L. balthica* and *Marenzelleria* spp., with only a scattering of other species present, while 3 and 5 had roughly 1/3 of *L. balthica*, *Marenzelleria* spp. and *M. affinis* each. This seems to indicate less stressful conditions for macrofauna on stations 3 and 5. There was also a significant difference in individual abundances between the two groups, although station 1 was also very nearly significantly different from station 6. This could be related to the larger grain size and related factors on stations 1 and 6, as locations with higher grain size are known to have a higher individual abundance (Gammal et al., 2019). The CCA clearly showed the same split between the stations, with stations 3 and 5 clustering near the higher sedimentary organic carbon, deeper, lower bottom-water oxygen stations while stations 1 and 6 were clearly on the lower organic carbon, shallower, higher oxygen concentration side of the gradient (Figure 9). Stations 1 and 6 also had a larger grain size, lower sediment clay content and higher C/N ratio, as expected from stations closer to shore (Appendix Table 1). Curiously, despite being in the lower organic carbon end of the gradient in CCA, in reality they nevertheless had slightly higher sediment organic carbon, possibly due to the vicinity of land and the incoming more resilient plant matter, and very slightly lower bottom water $O_2$ than stations 3 and 5 (Figure 9, Appendix Table 1). They also had a higher PLI in 2016, when sediment metal concentrations were analysed (Appendix Table 1). *M. affinis* is known to be sensitive to hypoxia and sediment $H_2S$, although in this case neither can easily explain their absence from stations 1 and 6 (Rousi et al., 2013). No clear, plausible explanation is provided by their known interactions with other species (predatory or competitive), either. However, *M. affinis* are known to be very sensitive to environmental stress, so their absence could indicate a higher level of environmental stress closer to the shore (Leonardsson et al. (2009)). It is also curious that this difference was only seen during the summer, as in the autumn stations 3 and 5 were significantly different from one another, while 5 and 6 were not. It is possible that the difference is more evident due to some seasonal effect of the heavy metals or some other factor that was not evident in the autumn samples, perhaps related to the macrofaunal life cycles.

The lengths and wet weights of *L. balthica* were also analysed, but only the summer 2017 wet weights showed significant differences between stations, with higher individual weights observed on the station furthest away from the shore (Figure 7, Figure 8). The difference was possibly caused by the settling of a new cohort in near-shore areas. This relatively small response was unexpected, since common effects of heavy metal pollution include both repressed growth and decrease in fertility and / or survival of the young

life stages (Bryan & Langston, 1992). However, *L. balthica* is known to be highly tolerant of environmental pollution and makes for a poor indicator species of pollution and environmental stress (Bryan & Langston, 1992). Its measurements were used in this study since it was the only species that was present on all stations, but this may have been a mistake.

## 4.2 Macrofaunal indices as indicators of heavy metal contamination

The tested macrofaunal indices did not detect all the differences between the sampling stations that were observed in the macrofaunal community data. H' differed most from the community analyses, finding none of the differences indicated by PERMANOVA tests in either year (Table 3). This seems to indicate that there were no significant species diversity differences between stations, either because heavy metal pollution did not influence species diversity or because some other environmental variable was obscuring the differences. The first case is unlikely, as multiple studies have found a significant effect of heavy metals on species diversity (Mucha et al., 2003; Neira et al. , 2015; Ryu et al., 2011). It is more likely that some other factor, in this case the coarser sediment on station 2, increased the species diversity and individual abundances there and made the possible effect of heavy metals undetectable (Gammal et al., 2019). The reason the H' values ranked as "poor" across all stations is unclear: H' is known to be sensitive to variations of salinity, as species diversity in brackish water is lowered due to the low salinity excluding both fully marine or fully freshwater species, but the H' reference values were calculated specifically for the conditions along the Finnish coast (Perus et al., 2007). Both the minimum and maximum values of H' were slightly higher in the 2010's than those observed by Luotamo in the 70's in the vicinity of our sampling stations (approximately 0,39 to 0,65 in Luotamo's report), but this could, again, easily be caused by a number of factors such as differences in sieve mesh size, species identification or timing of the sampling (Luotamo, 1974). BQI picked up some of the differences detected by the PERMANOVA, namely between station 2 and the rest in autumn 2016, and split the stations into the groups 1 and 6, and 3 and 5 in summer 2017 (Table 3). This is likely because BQI is not based on species diversity, rather based on environmental stress tolerances and abundances of the specific species with well-known ecology. The species found on the polluted station 2 were tolerant of environmental stress, even though their number was higher than on other stations (Leonardsson et al., 2009). This means that compared to H', BQI is less likely to be distracted by changes in sediment type or other environmental variables between sampling stations. However, it is still known to be sensitive to multiple types of environmental stress, whether anthropogenic or natural (stress caused by low salinity, for example), so a low BQI is not automatically an indication of human-induced stress (Zettler et al., 2007). BBI was something of a middle ground, not indicating any significant differences between stations in either year but coming close enough to be picked up by the Kruskal-Wallis test in summer 2017. This in-between behaviour of BBI might be due to the fact that the values of H' and BQI are weighted equally when calculating BBI, meaning that its values would end up somewhere between the two (Villnäs et al., 2015).

The macrofaunal indices correlated with one another to some degree. In case of BBI this was not surprising, as both H' and BQI are included in its formula and thus expected to correlate with the end result, but the correlation between H' and BQI shows that there is at least some connection between species diversity and the estimated ecological status of the community. However, this connection was not easily apparent in Figure 11, and became even more muddled when PLI was compared to the three indices. Despite correlating with one another, the indices differed widely in their connection to PLI. H' had no apparent connection to the sediment heavy metal concentration, probably due to the influence of the coarser sediment on species diversity (Figure 4, Figure 11, Appendix Table 1). BQI, however, seemed to have some negative connection to PLI, possibly due to the high stress tolerance of the species found on station 2

(Leonardsson et al., 2009; Villnäs et al., 2015). The muted, non-significant correlation of BBI and PLI might, again, have been related to the fact that H' and BQI are both included in calculating BBI, thus setting its values somewhere between the two (Villnäs et al., 2015).

Contrary to existing literature, no connection between the macrofaunal indices and either bottom-water oxygen concentration or salinity was found in our results (Villnäs et al., 2015). This is most likely because in our study the salinity varied little between stations, and the bottom-water oxygen concentrations did not reach hypoxia levels (as defined in Conley et al. (2011)) on any station. It is interesting that BQI seems to correlate with pore water $NH_4$, but as the Spearman's rank correlation tests had no correction applied to them, this could just be a side effect of testing multiple variables for correlations.

## 4.3 Comparison of sampling methods: GEMAX vs. van Veen grab

The data from comparisons between sampling methods was somewhat contradictory. Looking at the data itself, it seemed like on average the individual density per $m^2$ was slightly higher for GEMAX than the van Veen grab, but there was slightly more variability between replicate samples in the GEMAX, while the van Veen samples were more uniform (Figure 12). The different methods mostly picked up the same species, but in slightly different proportions. This could be due to the difference in sampled area, especially if the macrofaunal community has a heterogenous, patchy distribution, or if some of the sampled species are rare. In these cases the larger sampling area of the van Veen grab could offer a more even view of the macrofaunal community, an idea supported by Lampadariou et al. (2005). This is contrary to the findings of Souza & Barros (2014), although it should be noted that in their case the van Veen grab only sampled an area of 0,05 $m^2$ and a volume of 3,2 l while their core samples were composite samples totalling the area of 0,024 $m^2$ and 3,6 l, meaning that in their case the core samples actually had a larger volume than the van Veen grab. It is also interesting that all the species that were more common in the van Veen grab samples were soft-bodied and wormlike, even though the grab captures more material, and might therefore more easily damage the more delicate organisms during the sample sieving process. Statistically, neither total abundance data nor relative macrofaunal abundances indicated a significant difference between the sampling methods.

However, if the PERMANOVA was done using the standardised macrofaunal abundance data, a significant difference was detected between methods in autumn 2016. This could mean that the effect of the sampling gear is not large enough to be detected by either the total abundances or the distribution of species, but becomes detectable when the two factors are combined. It should also be noted that due to the processing time and effort required by the large van Veen samples, our data set only included three comparisons with five replicates each, which might not be enough to detect a significant but minor difference. It is unclear why the season makes a difference, although with only three station comparisons to draw from this could just be the result of random chance.

The three macrofaunal indices, which also combined information from both abundances and community composition, showed a significant difference between the methods. There was clearly more scatter in the GEMAX values, while the van Veen values grouped close together: a result consistent with the possibly patchy individual distribution in the sediment (Figure 15). The idea of patchiness was further supported by the GEMAX samples also estimating a lower average diversity or ecological status. When tested by station, both BQI and BBI even showed significant differences between methods summer 2017 station 1, where the standardised macrofaunal PERMANOVA detected no difference.

Overall there was no consistency in the GEMAX – van Veen grab comparison results. It is possible that the set of fifteen comparisons from three station pairs is just too small to tell whether or not a difference exists. Since even the literature on the topic is contradictory, it might be prudent to conduct more studies on the topic before coming to any final conclusion (Lampadariou et al., 2005; Souza & Barros, 2014). For now, if the time and resources permit, van Veen grab might be the safer choice, but there is no definitive proof that the smaller GEMAX samples are not as representative of the macrofaunal community.

# 5. Conclusions

Some environmental stress is evident in the macrofaunal communities, especially at station 2 (sampled in autumn 2016 only), but the cause of it is uncertain. The stress on station 2 could be due to the high heavy metal concentrations, but this cannot be confirmed by correlation only. The stress on autumn 2016 stations 3 and 4 is more likely to be caused by the high pore water concentrations of $H_2S$ related to the possible lack of oxygen within the sediment. The cause of the higher stress on macrofauna on stations 1 and 6 in summer 2017 is also unclear, but it seems related to PLI and the variables that correlate with it. It is possible that the effects of sedimentary heavy metals are more clearly seen during the summer, either due to seasonal changes in sediment chemistry or macrofaunal life cycles, but that cannot be confirmed or denied based on the data available. In future studies a clearer gradient of sedimentary heavy metal concentrations with multiple sampling stations located in heavily polluted areas would make it easier to separate the influence of heavy metals from other environmental variables. The relative similarity of sampling stations in terms of other environmental variables would be ideal but can be impossible to achieve in the field. Another option would be to test the effects of heavy metals in a more controlled laboratory environment, but that risks reducing the complexity of the macrofaunal community and making it more susceptible to the heavy metals as well as introducing potential artificial errors.

Of the macrofaunal indices, H' was the least successful in detecting the differences between the sampling stations, probably due to interference from other environmental factors like the coarser sediment. The differences detected by BQI resembled those found by the PERMANOVA: it was clearly picking up some differences in environmental status between the stations, both between the polluted station 2 and the rest in 2016, and between the near-shore and further-away stations in summer 2017. While BBI did not detect the differences between the sampling stations, it was statistically quite close to significance. A higher number of samples might have made a difference. It is recommended that studies calculate more than one macrofaunal index to evaluate environmental status in a sampling site to get a more comprehensive view of the conditions.

The comparisons between sampling methods gave contradictory results, possibly due to a too small data set. There were some indications that a van Veen grab taking a larger volume sample might be the more consistent sampling method if the macrofaunal community is assumed to be spatially heterogenous. However, this would drastically increase the time and effort required to process samples, so it might not be a viable option in all cases (Lampadariou et al., 2005). As the results from GEMAX samples were not conclusively found to differ from van Veen samples, it remains a usable alternative in macrofaunal sampling. However, if sampling with GEMAX one should keep in mind that in a spatially heterogenous macrofaunal community it is possible to under- or overestimate the individual density and miss some of the tightly clustered species, unless a sufficient number of replicates is taken.

# 6. Acknowledgements

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# Appendix 1: Environmental conditions

This data was collected under the research project "Next Generation Tool for Ecosystem Assessment" and given to my use by Karoliina Koho.

## Sampling

At each station two GEMAX cores were taken for geochemical analyses. One of the cores was used for analyses of the sediment and pore water composition, including sediment concentrations of selected heavy metals (As, Cd, Cr, Cu, Ni, Pb, Zn), sedimentary organic carbon ($C_{org}$), sedimentary total nitrogen content ($N_{tot}$), sediment isotopic composition of organic carbon ($\delta^{13}C_{org}$), sediment grain size (top 1 cm), pore water nitrate ($NO_3^-$), pore water ammonium ($NH_4^+$) and pore water hydrogen sulphide ($H_2S$). Another core was used for analyses of dissolved oxygen in the sediment.

Pore water sampling and analyses of $NO_3^-$, $NH_4^+$ and $H_2S$, sedimentary $C_{org}$ and $N_{tot}$ content, and $\delta^{13}C_{org}$ were carried out as described in Jilbert et al. (2018). Porewater oxygen profiles were measured with Unisense microsensor (OX-100) that was two-point calibrated, first in 100% air-saturated filtered sea water collected from the study site, and then in anoxic solution containing sodium ascorbate and NaOH (both at 0.1M). Sediment grain size was measured at Royal Netherlands Institute for Sea Research with a with a Coulter laser diffraction particle sizer (LS13320). The concentrations of heavy metals in sediment were determined at Geo Lab, Utrecht University, the Netherlands, using a Thermo Element 2 ICP-MS. Precision and accuracy in all cases were within 5% as determined by replicate analyses with reference to in-house standards.

Water column properties, including temperature and salinity, were also recorded at each station with a conductivity temperature depth profiler (CTD). Bottom-water oxygen content was determined using standard Winkler titration (Winkler, 1888).

The environmental conditions on each station are shown in Appendix Table 1. The temperature anomalies at stations 5 and 6 during the autumn 2016 are most likely due to an autumn storm and the beginning of snowfall between sampling days.

## Environmental variables

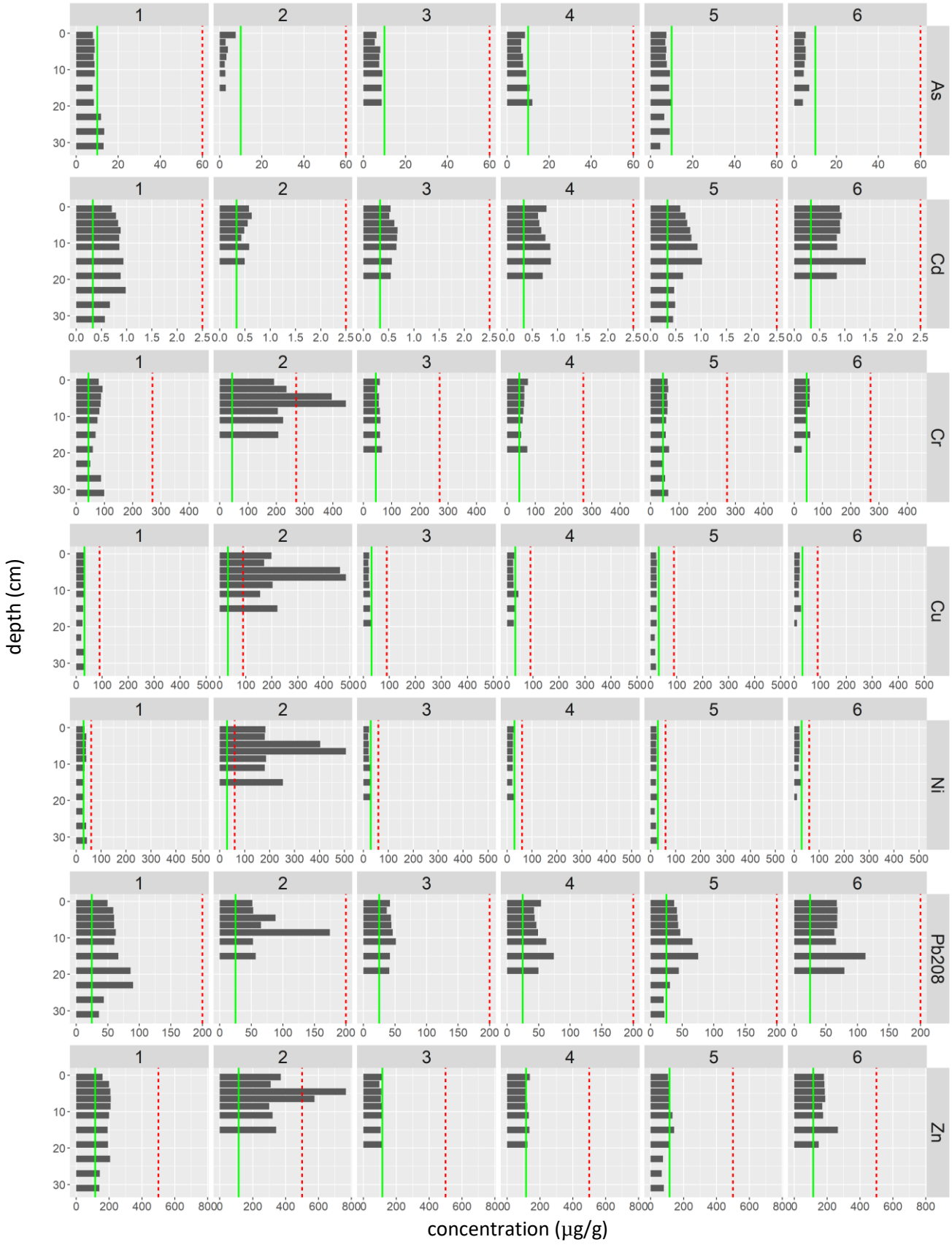
There is a gradient of increasing depth and sediment clay content and decreasing grain size and C/N ratio from shore outwards in both seasons (Appendix Table 1). In sediment carbon content we see an increasing outwards gradient during autumn and a decreasing one during summer, although autumn 2016 station 6 does not fit in this pattern, indicating increased sedimentation of carbon there during autumn. No clear gradient can be found in the proportion of $^{13}C$ in the top 1 cm of sediment, sediment pore water $NH_4$ or $H_2S$, or bottom water $O_2$ concentration, temperature or salinity.

## Metal concentrations

The sediment concentration profiles for potentially harmful metals according to the Finnish Ministry of Environment (Suomen ympäristöministeriö, 2004), along with the natural background (Kemppainen, 2000) and high (potentially toxic) concentrations (Suomen ympäristöministeriö, 2004), are shown in Appendix Figure 1. Of the metals analysed, Cd and Pb showed slightly elevated concentrations on most stations, while Cr, Cu, Ni and Zn reached potentially harmful levels at station 2 and followed the baseline elsewhere. Tin (Sn) can also be potentially toxic to macrofauna, but it was not included in the sediment guidelines cited, and thus the measured concentrations were not comparable to reference values.

| sampling time | station | water depth | grain | clay | C_1cm | 13C_1cm | CN_1cm | NH4_inv_PW | HS_inv_PW | O2_BW | temp_BW | sal_BW | PLI |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | (m) | (µm) | (%) | (wt %) | (del) | (mol) | (µmol/cm$^2$) | (µmol/cm$^2$) | (µmol/l) | (°C) | | |
| autumn 2016 | 1 | 16,5 | 9,2 | 24,3 | 4,8 | -23,0 | 9,1 | 2,60 | 0,03 | 250,6 | 7,2 | 5,4 | 1,54 |
| autumn 2016 | 2 | 8,0 | 584,4 | 0,8 | 0,7 | -22,4 | 26,8 | 1,06 | 0,01 | 328,4 | 7,5 | 5,5 | 3,18 |
| autumn 2016 | 3 | 22,0 | 4,7 | 46,0 | 4,9 | -22,8 | 9,1 | 1,97 | 1,93 | 207,3 | 7,6 | 5,6 | 1,07 |
| autumn 2016 | 4 | 22,0 | 6,5 | 39,9 | 5,1 | -21,7 | 8,9 | 2,05 | 1,59 | 186,3 | 7,5 | 5,5 | 1,23 |
| autumn 2016 | 5 | 22,8 | 6,3 | 38,0 | 6,8 | -21,4 | 8,9 | 1,50 | 0,32 | 232,9 | 4,2 | 5,4 | 1,17 |
| autumn 2016 | 6 | 15,0 | 8,4 | 35,7 | 6,1 | -22,3 | 11,1 | 2,18 | 0,43 | 165,4 | 3,4 | 5,3 | 1,23 |
| summer 2017 | 1 | 15,7 | 16,9 | 28,3 | 6,0 | -21,5 | 15,9 | 0,92 | 1,02 | 208,4 | 9,3 | 6,2 | 1,54 |
| summer 2017 | 3 | 22,4 | 5,0 | 43,7 | 5,6 | -21,7 | 10,0 | 2,69 | 0,58 | 214,3 | 9,0 | 6,2 | 1,07 |
| summer 2017 | 5 | 27,9 | 4,8 | 45,0 | 5,3 | -21,7 | 8,8 | 1,37 | 0,67 | 219,5 | 8,7 | 6,3 | 1,17 |
| summer 2017 | 6 | 15,1 | 9,6 | 33,3 | 7,8 | -21,0 | 14,2 | 1,43 | 0,21 | 205,3 | 10,0 | 6,1 | 1,23 |

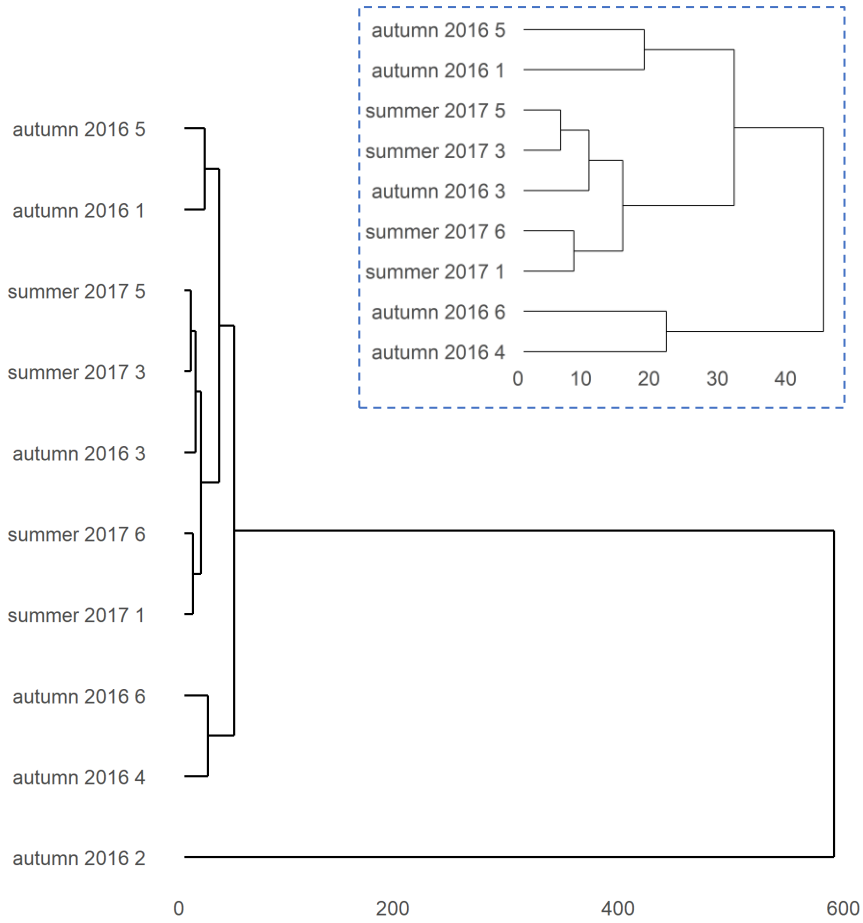
*Appendix Table 1. The environmental conditions on the sampling sites. depth = water depth, clay = sediment clay content, C_1cm = amount of organic carbon in the top 1 cm of sediment, 13C_1cm = portion of $^{13}C$ in the top 1 cm of sediment, CN_1cm = molar C/N ratio in the top 1 cm of sediment, NH4_inv_PW = sediment pore water $NH_4$ inventory, HS_inv_PW = sediment pore water $H_2S$ inventory, O2_BW = bottom water $O_2$ concentration, temp_BW = bottom water temperature, sal_BW = bottom water salinity, PLI = Pollution Load Index.*




*Appendix Figure 1. Sediment concentration profiles of potentially harmful metals. Green line = natural background concentration, red line = high (potentially toxic) concentration.*

## Environmental data analysis

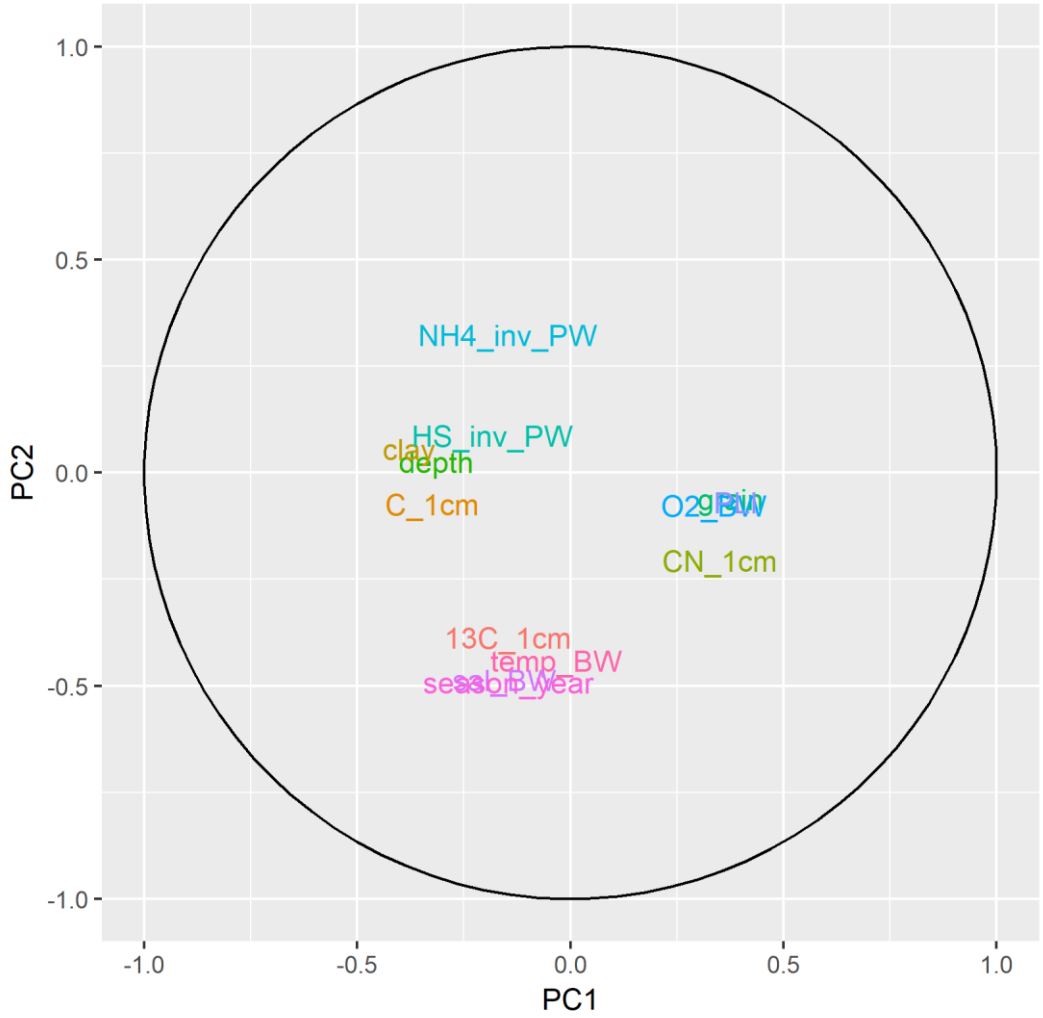
A distance matrix was calculated for the stations based on the environmental variables and the results plotted as a dendrogram (Appendix Figure 2). Environmentally most similar to one another are the summer 2017 stations 3 and 5, and 1 and 6. Interestingly summer 2016 station 3 is more similar to summer 2017 stations 3 and 5 than they are to the summer 2017 stations 1 and 6. We can also see that autumn 2016 stations 1 and 5 are more similar to the previously described cluster than they are to the last two autumn 2016 stations, 4 and 6. Finally, station 2 is very different from the other stations in terms of environmental variables. Thus, in terms of environmental variables, the dendrogram can be cut into four clusters along the dissimilarity of 20 on the scale of Appendix Figure 2: summer 2017 stations plus autumn 2016 station 3, autumn 2016 stations 1 and 5, autumn 2016 stations 4 and 6, and autumn 2016 station 2 (Appendix Figure 2).




*Appendix Figure 2. Dendrogram of the dissimilarities between sampling stations in both years. Zoom-in on the structure without station 2 In the upper right corner.*

Environmentally station 2 stood out as exceptional in almost every way (Appendix Figure 2). It was closest to the shore and the only station with water depth less than 10 meters and had the average grain size 83 times larger than the other stations (Figure 1, Appendix Table 1). The sediment proportions of clay and organic carbon on station 2 were extremely low compared to the other stations, the C/N ratio was double that of the other stations, the bottom water $O_2$ concentrations were high and the PLI was triple the average of the other stations (Appendix Table 1). The other stations were more similar to one another, although there was still greater variation among the autumn 2016 stations than there was within the summer 2017 stations (Appendix Figure 2). Greatest variations between stations (excluding station 2) in autumn 2016 were observed in depth, sediment clay and pore water $H_2S$ content, bottom water $O^2$ concentrations and temperature (Appendix Table 1). The observed temperature difference of several degrees on stations 5 and 6 is known to be an artefact, as the water temperatures were affected by a snowstorm between the sampling of stations 1-4 and stations 5-6. The autumn 2016 station 3 bore a close resemblance to summer 2017 stations, partially due to variables that are unlikely to change between years, like grain size and clay content, but also in terms of more seasonal variables like sedimentary organic carbon content and C/N ratio. In summer 2017 the stations were split in two groups, stations 1 and 6, and stations 3 and 5, based on differences depth, grain size, clay content and sediment C/N ratio between the groups (Appendix Figure 2, Appendix Table 1).

Distance matrices were calculated for the environmental variables, which were then were plotted with PCA to find correlations between parameters (Appendix Figure 3). One parameter was chosen from each group of correlating parameters to reduce correlations between parameters in CCA, since this would result in high variance inflation factors (VIFs) and distort the results.




*Appendix Figure 3. PCA of environmental variables on the stations. Pore water $NH_4$ inventory (NH4_inv_PW), pore water $H_2S$ inventory (HS_inv_PW), sediment clay content (clay), sediment grain size (grain), water depth (depth), sediment organic carbon content in the top 1 cm (C_1cm), sediment C/N ratio in the top 1 cm (CN_1cm), portion of $^{13}C$ in the top 1 cm (13C_1cm), bottom-water $O_2$ content (O2_BW), bottom-water salinity (sal_BW), season_year = sampling season and year, bottom water temperature (temp_BW) and PLI (Pollution Load Index).*

As Appendix Figure 3 shows, the environmental variables grouped into four distinct clusters based on how similarly they behaved. The variables chosen to represent each of the clusters were:

1. NH4_inv_PW
2. C_1cm representing depth, HS_inv_PW, clay and C_1cm
3. PLI representing O2_BW, grain and C/N_1 cm and PLI
4. season_year representing 13C_1 cm, temp_BW, sal_BW and season_year

The proportion of organic carbon in the top 1 cm of sediment seemed to be linked to water depth, pore water $H_2S$ inventory and sediment clay content, but contrary to Bryan & Langston (1992), had nothing to do with the sediment heavy metal concentrations. This, again, shows the influence of the very unusual station 2. Sampling year and season were connected to the proportion of $^{13}C$ in the top 1 cm of sediment,

water temperature and salinity, all of which had remarkably little non-artificial variation within the season and year, meaning they represent more static variables unlikely to contribute to the within-year differences between stations. The sediment heavy metal content, represented by PLI, appeared linked to bottom-water $O^2$ concentration, sediment grain size and C/N ratio. The links between these variables could, however, be somewhat artificial. While bottom-water $O^2$ concentration and sediment grain size are connected through lower consumption of bottom-water O2 when grain size is large (less fine-grained organic matter to decompose), the most apparent reason for the connection between them, the C/N ratio and the PLI comes down to circumstances. While the factory harbour was understandably the most polluted area, it was also the one closest to shore, and thus most likely to get allochthonous inputs from land, which would affect the C/N ratio. The sediment in the harbour would also likely be disturbed by regular dredging or traffic, which could explain the grain size difference. The pore water $NH_4$ inventories seemed behave separately from all other variables.

## Appendix 2: Macrofaunal data

| method | season year | station | sample | *L. balt* | *M. are* | *Cera* | *M. edu* | *H. div* | *Mare* | *Pygo* | *Mana* | *Hydro* | *Potam* | *Oligo* | Chiro | *Ostra* | *Gamm* | *M. aff* | *Ponto* | *H. spin* | *C. obs* | *Mysis* | *B. sar* | *Prost* | *Lima* | Cope | *S. ent* |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| GEMAX | autumn 2016 | 1 | 1 | 1100 | 0 | 0 | 0 | 0 | 157 | 0 | 0 | 472 | 629 | 157 | 157 | 943 | 157 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| GEMAX | autumn 2016 | 1 | 2 | 1415 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 472 | 943 | 0 | 0 | 629 | 0 | 0 | 0 | 157 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| GEMAX | autumn 2016 | 1 | 3 | 5187 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 314 | 157 | 157 | 472 | 629 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| GEMAX | autumn 2016 | 1 | 4 | 4087 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 314 | 157 | 0 | 943 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| GEMAX | autumn 2016 | 1 | 5 | 629 | 0 | 0 | 0 | 0 | 157 | 0 | 0 | 0 | 0 | 0 | 314 | 786 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| GEMAX | autumn 2016 | 2 | 1 | 6445 | 0 | 0 | 0 | 0 | 4244 | 0 | 0 | 1257 | 1886 | 1729 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 157 | 0 |
| GEMAX | autumn 2016 | 2 | 2 | 4087 | 0 | 0 | 0 | 0 | 3301 | 314 | 0 | 157 | 1415 | 2201 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 314 | 0 |
| GEMAX | autumn 2016 | 2 | 3 | 5973 | 0 | 157 | 0 | 157 | 4244 | 629 | 0 | 314 | 786 | 1886 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| GEMAX | autumn 2016 | 2 | 4 | 5187 | 0 | 0 | 0 | 0 | 4244 | 0 | 0 | 629 | 1100 | 2358 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 157 | 0 |
| GEMAX | autumn 2016 | 2 | 5 | 1415 | 157 | 0 | 0 | 0 | 4558 | 0 | 0 | 157 | 472 | 786 | 0 | 0 | 0 | 0 | 0 | 0 | 157 | 0 | 0 | 0 | 0 | 0 | 0 |
| GEMAX | autumn 2016 | 3 | 1 | 1886 | 0 | 0 | 0 | 0 | 157 | 0 | 0 | 0 | 0 | 0 | 0 | 157 | 0 | 157 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| GEMAX | autumn 2016 | 3 | 2 | 3301 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 472 | 0 | 314 | 314 | 157 | 157 | 0 | 157 | 0 | 157 | 0 | 0 | 0 | 0 | 0 |
| GEMAX | autumn 2016 | 3 | 3 | 2672 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 314 | 0 | 0 | 314 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| GEMAX | autumn 2016 | 3 | 4 | 1729 | 0 | 0 | 0 | 0 | 157 | 0 | 0 | 472 | 314 | 0 | 0 | 314 | 0 | 314 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| GEMAX | autumn 2016 | 3 | 5 | 3144 | 0 | 0 | 0 | 0 | 314 | 0 | 0 | 157 | 1415 | 0 | 0 | 157 | 0 | 157 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| GEMAX | autumn 2016 | 4 | 1 | 2829 | 0 | 0 | 0 | 0 | 629 | 0 | 0 | 314 | 0 | 0 | 157 | 157 | 0 | 157 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| GEMAX | autumn 2016 | 4 | 2 | 1257 | 0 | 0 | 0 | 0 | 157 | 0 | 0 | 0 | 629 | 0 | 157 | 157 | 0 | 314 | 0 | 0 | 0 | 0 | 157 | 0 | 0 | 0 | 0 |
| GEMAX | autumn 2016 | 4 | 3 | 2515 | 0 | 0 | 0 | 0 | 157 | 0 | 0 | 157 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| GEMAX | autumn 2016 | 4 | 4 | 943 | 0 | 0 | 0 | 0 | 629 | 0 | 0 | 0 | 0 | 0 | 157 | 157 | 0 | 157 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| GEMAX | autumn 2016 | 4 | 5 | 2201 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 157 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| GEMAX | autumn 2016 | 5 | 1 | 943 | 0 | 0 | 0 | 0 | 157 | 0 | 0 | 0 | 0 | 0 | 157 | 472 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| GEMAX | autumn 2016 | 5 | 2 | 1257 | 0 | 0 | 0 | 0 | 472 | 0 | 0 | 157 | 0 | 0 | 0 | 786 | 0 | 157 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 314 | 0 |
| GEMAX | autumn 2016 | 5 | 3 | 1100 | 0 | 0 | 0 | 0 | 157 | 0 | 0 | 0 | 0 | 0 | 0 | 1100 | 0 | 943 | 0 | 0 | 0 | 0 | 157 | 0 | 0 | 0 | 0 |
| GEMAX | autumn 2016 | 5 | 4 | 1415 | 0 | 0 | 0 | 0 | 472 | 0 | 0 | 0 | 0 | 0 | 157 | 786 | 0 | 629 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| GEMAX | autumn 2016 | 5 | 5 | 2043 | 0 | 0 | 0 | 0 | 629 | 0 | 0 | 0 | 0 | 0 | 0 | 157 | 0 | 472 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

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|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| GEMAX | autumn 2016 | 6 | 1 | 943 | 0 | 0 | 314 | 0 | 0 | 0 | 0 | 943 | 786 | 157 | 0 | 2201 | 157 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| GEMAX | autumn 2016 | 6 | 2 | 1257 | 0 | 0 | 629 | 0 | 0 | 0 | 0 | 314 | 786 | 314 | 157 | 2358 | 157 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 157 | 0 |
| GEMAX | autumn 2016 | 6 | 3 | 2515 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 629 | 157 | 157 | 0 | 1257 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| GEMAX | autumn 2016 | 6 | 4 | 1572 | 0 | 0 | 0 | 0 | 157 | 0 | 0 | 157 | 0 | 0 | 157 | 314 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| GEMAX | autumn 2016 | 6 | 5 | 3144 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 314 | 314 | 629 | 0 | 1257 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

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|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| GEMAX | summer 2017 | 1 | 1 | 1257 | 0 | 0 | 0 | 0 | 1415 | 0 | 0 | 0 | 0 | 0 | 0 | 314 | 0 | 157 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| GEMAX | summer 2017 | 1 | 2 | 1886 | 0 | 0 | 0 | 0 | 2672 | 0 | 0 | 0 | 0 | 0 | 314 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| GEMAX | summer 2017 | 1 | 3 | 1729 | 0 | 0 | 0 | 0 | 2358 | 0 | 0 | 0 | 0 | 0 | 0 | 314 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| GEMAX | summer 2017 | 1 | 4 | 1572 | 0 | 0 | 0 | 0 | 1729 | 0 | 0 | 0 | 0 | 157 | 157 | 157 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| GEMAX | summer 2017 | 1 | 5 | 2672 | 0 | 0 | 0 | 0 | 2201 | 0 | 0 | 0 | 1257 | 0 | 157 | 157 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 157 |
| GEMAX | summer 2017 | 3 | 1 | 1572 | 0 | 0 | 0 | 0 | 1257 | 0 | 0 | 0 | 157 | 0 | 157 | 472 | 0 | 943 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| GEMAX | summer 2017 | 3 | 2 | 2358 | 0 | 0 | 0 | 0 | 1100 | 0 | 0 | 0 | 314 | 0 | 0 | 314 | 0 | 943 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| GEMAX | summer 2017 | 3 | 3 | 786 | 0 | 0 | 0 | 0 | 1729 | 0 | 0 | 0 | 0 | 0 | 0 | 157 | 0 | 943 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| GEMAX | summer 2017 | 3 | 4 | 1886 | 0 | 0 | 0 | 0 | 943 | 0 | 0 | 0 | 157 | 0 | 0 | 314 | 0 | 1257 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| GEMAX | summer 2017 | 3 | 5 | 1886 | 0 | 0 | 0 | 0 | 1572 | 0 | 0 | 0 | 157 | 0 | 0 | 0 | 0 | 629 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| GEMAX | summer 2017 | 5 | 1 | 786 | 0 | 0 | 0 | 0 | 1572 | 0 | 0 | 0 | 0 | 0 | 0 | 314 | 0 | 1257 | 157 | 0 | 0 | 0 | 0 | 0 | 157 | 0 |
| GEMAX | summer 2017 | 5 | 2 | 1100 | 0 | 0 | 0 | 0 | 1572 | 0 | 0 | 0 | 0 | 0 | 157 | 472 | 0 | 1886 | 157 | 0 | 0 | 0 | 157 | 0 | 0 | 0 | 0 |
| GEMAX | summer 2017 | 5 | 3 | 1572 | 0 | 0 | 0 | 0 | 786 | 0 | 0 | 0 | 0 | 0 | 157 | 629 | 0 | 1100 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| GEMAX | summer 2017 | 5 | 4 | 1886 | 0 | 0 | 0 | 0 | 1257 | 0 | 0 | 0 | 0 | 0 | 157 | 314 | 0 | 1100 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| GEMAX | summer 2017 | 5 | 5 | 1572 | 0 | 0 | 0 | 0 | 1415 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1100 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| GEMAX | summer 2017 | 6 | 1 | 2515 | 0 | 0 | 0 | 0 | 3144 | 0 | 0 | 0 | 314 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 157 | 0 | 0 | 0 |
| GEMAX | summer 2017 | 6 | 2 | 3144 | 0 | 0 | 0 | 0 | 3301 | 0 | 157 | 0 | 157 | 157 | 157 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 157 | 0 | 0 | 0 |
| GEMAX | summer 2017 | 6 | 3 | 4087 | 0 | 0 | 157 | 0 | 2201 | 0 | 0 | 157 | 157 | 472 | 157 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 157 | 0 | 0 | 0 |
| GEMAX | summer 2017 | 6 | 4 | 3772 | 0 | 0 | 0 | 0 | 4244 | 0 | 0 | 0 | 157 | 157 | 157 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 157 | 0 | 0 | 0 | 0 |
| GEMAX | summer 2017 | 6 | 5 | 2829 | 0 | 0 | 0 | 0 | 1729 | 0 | 0 | 157 | 0 | 472 | 157 | 314 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 157 | 0 | 0 | 0 |

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|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| grab | autumn 2016 | 1 | 1 | 1958 | 0 | 0 | 0 | 0 | 47 | 0 | 0 | 70 | 225 | 109 | 171 | 256 | 0 | 16 | 0 | 8 | 0 | 0 | 0 | 0 | 0 | 0 |
| grab | autumn 2016 | 1 | 2 | 1344 | 0 | 0 | 0 | 0 | 451 | 0 | 0 | 62 | 241 | 70 | 591 | 311 | 0 | 16 | 0 | 8 | 0 | 0 | 0 | 0 | 0 | 0 |
| grab | autumn 2016 | 1 | 3 | 1235 | 0 | 0 | 0 | 0 | 117 | 0 | 0 | 124 | 186 | 117 | 202 | 303 | 0 | 8 | 0 | 16 | 0 | 8 | 0 | 0 | 0 | 8 |
| grab | autumn 2016 | 1 | 4 | 1096 | 0 | 0 | 0 | 0 | 8 | 0 | 0 | 70 | 218 | 132 | 70 | 233 | 0 | 0 | 0 | 8 | 0 | 0 | 0 | 0 | 0 | 0 |
| grab | autumn 2016 | 1 | 5 | 1935 | 0 | 0 | 0 | 0 | 54 | 0 | 0 | 78 | 326 | 109 | 171 | 272 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| grab | autumn 2016 | 5 | 1 | 420 | 0 | 0 | 0 | 0 | 272 | 0 | 0 | 0 | 0 | 0 | 31 | 264 | 0 | 319 | 0 | 8 | 0 | 0 | 16 | 0 | 0 | 8 |
| grab | autumn 2016 | 5 | 2 | 901 | 0 | 0 | 0 | 0 | 272 | 0 | 0 | 23 | 8 | 0 | 93 | 443 | 0 | 225 | 0 | 31 | 0 | 8 | 31 | 0 | 0 | 0 |
| grab | autumn 2016 | 5 | 3 | 987 | 0 | 0 | 0 | 0 | 210 | 0 | 0 | 16 | 0 | 0 | 140 | 637 | 0 | 233 | 0 | 16 | 0 | 0 | 16 | 0 | 0 | 8 |
| grab | autumn 2016 | 5 | 4 | 785 | 0 | 0 | 0 | 0 | 264 | 0 | 0 | 0 | 8 | 0 | 117 | 591 | 0 | 233 | 0 | 39 | 0 | 0 | 16 | 0 | 0 | 0 |
| grab | autumn 2016 | 5 | 5 | 1064 | 0 | 0 | 0 | 0 | 264 | 0 | 0 | 0 | 0 | 0 | 93 | 544 | 0 | 249 | 0 | 23 | 0 | 0 | 8 | 0 | 0 | 0 |
| grab | summer 2017 | 1 | 1 | 2145 | 8 | 0 | 0 | 0 | 2401 | 0 | 0 | 0 | 109 | 0 | 225 | 389 | 0 | 93 | 0 | 31 | 0 | 8 | 0 | 0 | 0 | 0 |
| grab | summer 2017 | 1 | 2 | 1943 | 8 | 0 | 16 | 0 | 1857 | 0 | 0 | 47 | 280 | 23 | 109 | 466 | 0 | 186 | 0 | 8 | 0 | 0 | 8 | 0 | 0 | 16 |
| grab | summer 2017 | 1 | 3 | 3854 | 0 | 0 | 0 | 0 | 1538 | 0 | 0 | 16 | 280 | 70 | 171 | 54 | 0 | 404 | 0 | 39 | 0 | 0 | 39 | 0 | 0 | 0 |
| grab | summer 2017 | 1 | 4 | 2401 | 0 | 0 | 0 | 0 | 1911 | 0 | 0 | 0 | 62 | 47 | 218 | 287 | 0 | 155 | 0 | 8 | 0 | 0 | 31 | 0 | 0 | 8 |
| grab | summer 2017 | 1 | 5 | 2277 | 0 | 0 | 0 | 0 | 2587 | 0 | 0 | 8 | 124 | 117 | 109 | 404 | 0 | 132 | 0 | 8 | 0 | 0 | 0 | 0 | 16 | 0 | 16 |

The data in this appendix is given as individuals / $m^2$ and contains both the 1 mm and the 0,5 mm sieve fractions.